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NEW OR OTHERWISE NOTEWORTHY APOCYNACEAE OF TROPICAL AMERICA. III[1]

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Peltastes Woodson, gen. nov. Apocynacearum (Echitoideae). Calyx majusculis profunde 5-partitus; laciniae foliaceae plus minusve inaequales margine imbricatae intus basi squamas minutas plus minusve numerosas uniforme gerentes. Corolla speciosissima magna infundibuliformis; tubus inferne latiuscule cylindricus dein circa medium inferiusve staminiger et in fauces aut conicas aut tubulatas aut campanulatas late dilatatus numquam constrictus; limbi laciniae 5 aequales oblique obovatae aestivatione dextrorsum convolutae. Stamina 5 omnino inclusa; antherae inter se adglutinatae et stigmati adplicatae anguste sagittatae dorso superne dense hirsutulae basi angustissime 2-auriculatae dimidia parte superiore ventro pollinigerae, sporangiis binis basi in appendiculas rigidas productis, pollinibus granulosis; filamenta ligulata perbrevia laxe pilosula. Ovarii carpella gemina basi distincta apice in stylo gracili producta, ovulis multis in quoque loculo pluriseriatim superpositis; stigma capitato-fusiforme apice obscure bipartitum. Nectarii glandulae 5 saepissime separatae vel basi paulo connatae. Fructus folliculares apocarpi crassiuscule tereti acuminati plus minusve falcati; semina numerosa fusiforme-subscaphoidea apice latiuscule rostrata ibique bene comosa.—Frutices lactescentes volubiles; folia opposita petiolata rigide membranacea vel coriacea eglandulosa peltata. Inflorescentia lateralis vel rarius subterminalis

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opposita paniculata rariusve subumbellata, bracteis foliaceis oppositis.

Peltastes peltatus (Vell.) Woodson, comb. nov.
Echites peltata Vell. Fl. Flum. 110. 1830; Icon. 3: *pl. 32*. 1827.
Stipecoma peltata (Vell.) Miers, Apoc. So. Am. 134. 1878.

Peltastes macrocalyx (Muell.-Arg.) Woodson, comb. nov.
Echites macrocalyx Muell.-Arg. in Martius, Fl. Bras. 6[1]: 160. 1860.
Stipecoma macrocalyx (Muell.-Arg.) Miers, loc. cit. 136. 1878.

Peltastes malvaeflorus Woodson, sp. nov., suffruticosa volubilis; ramis teretibus sat crassis juventate dense et minute ferrugineo-tomentulosis tandem glabratis cortice brunneis lutescentibus rimosis; foliis oppositis petiolatis peltatis rigide membranaceis ovatis vel ovato-oblongis apice abrupte acuminatis basi rotundatis 10–18 cm. longis 6–12 cm. latis supra juventate minute ferrugineo-tomentellis tandem glabratis subtus indumento simili ornatis; petiolis 2.5–4.0 cm. longis ut in folio vestitis; inflorescentiis paniculatis cymosis plus minus fasciculatis; pedunculis foliis ca. dimidia aequantibus 1–9-floris dense ferrugineo-tomentellis; pedicellis 1.5–2.0 cm. longis; bracteis foliaceis anguste oblongis ca. 1 cm. longis; calycis lobis foliaceis anguste oblongis abrupte acuminatis 1.0–1.5 cm. longis sparse ferrugineo-tomentellis; corollae speciosissimae gilvae inferne brunnescentis extus omnino glabrae tubo-proprio late cylindrico 0.75–1.25 cm. longo basi ca. 0.75 cm. diametro metiente ad apicem staminigero ibique abrupte dilatato faucibus campanulatis 2.0–2.5 cm. longis ostio ca. 2 cm. diametro metiente lobis late obovato-dolabriformibus 2.5–3.0 cm. longis patulis; antheris anguste sagittatis ca. 1 cm. longis; ovariis ovoideis ca. 0.4 cm. longis glabris; stigmate 0.2 cm. longo; nectarii glandulis ovoideis ovariis vix aequantibus; folliculis crassiusculis 20–25 cm. longis apice connatis glabris vel minutissime papillatis; seminibus 2.25 cm. longis parte dimidia superiore rostratis como dilute aurantiaco ca. 5 cm. longo.—BRAZIL: Parana: Valhinos, ad marginem silvae primaevae, Nov. 11, 1910, *P. Dusen 10851* (*in flore:* Mo. Bot. Garden Herbarium, TYPE, Herbarium Mus. Bot. Stockholm, duplicate); Rio Grande

do Sul: Silveira Martina, prope Santa Maria, in silva primaeva, March 6, 1893, *G. A. Malme 690* (*in fructu:* Herbarium Mus. Bot. Stockholm, COTYPE, Mo. Bot. Garden Herbarium, photograph).

This species is apparently widely established, having been collected upon numerous occasions in the Brazilian states of Parana, Sao Paulo, and Rio Grande do Sul, by Dusen, the Regnell Exploration parties of the Botanical Museum at Stockholm, and the botanists of the Expedition der Kaiserlichen Akademie der Wissenschaften in Wien. It differs from the closely related *P. peltatus*, for which it has been mistaken, in the larger corolla, the tube of which about half equals the throat, the proportionally shorter calyx-lobes, and the fewer-flowered inflorescence. The leaves of the latter species are also relatively larger than in *P. malvaeflorus*, and a slightly more coriaceous texture predominates. At present, true *P. peltatus* is known with certainty only from the Brazilian state of Minas Geraes.

Peltastes stemmadeniiflorus Woodson, sp. nov., suffruticosa volubilis; ramis subcompressis sat crassis juventate ferrugineo-tomentulosis maturitate glabratis; foliis oppositis petiolatis peltatis rigide membranaceis late ovatis apice abrupte acuminatis basi rotundatis 18–20 cm. longis 12–15 cm. latis supra glabratis subtus glabratis vel sparse ferrugineo-tomentellis; petiolis 7–9 cm. longis ut in folio vestitis; inflorescentiis paniculatis cymosis lateralibus oppositis; pedunculis petiolis vix dimidio aequantibus dense ferrugineo-tomentulosis ca. 7-floris; pedicellis 1.5 cm. longis ut in pedunculo vestitis; bracteis foliaceis glabratis oblongo-spathulatis pedicello ca. aequantibus; calycis lobis foliaceis late oblongo-spathulatis abrupte acuminatis 1.5–2.0 cm. longis extus glabratis inferne sparse tomentellis; corollae speciosissimae ut videtur flavae extus omnino glabrae tubo-proprio 2.25 cm. longo basi ca. 0.75 cm. diametro metiente faucibus gradatim dilatatis conico-campanulatis 2 cm. longis ostio ca. 1.5 cm. diametro metiente lobis late obovato-dolabriformibus acuminatis 2.0–2.5 cm. longis patulis; genitaliis ignotis; folliculis desiderantur.—PARAGUAY: in altaplanitie et declivibus, Sierra de Amambay, Dec. 1907, *T. Rojas 9838* (Herbarium Mus. Palat. Vindob., TYPE, Mo. Bot. Garden Herbarium, photograph).

The larger calyx-lobes, longer corolla tube-proper, and gradually dilating, conical-campanulate corolla-throat serve to distinguish *P. stemmadeniiflorus* from the neighboring *P. malvaeflorus* of southern Brazil.

Peltastes giganteus Woodson, sp. nov., suffruticosa volubilis; ramis teretiusculis sat crassis juventate ut videntur minute ferrugineo-tomentulosis maturitate certe glabratis; foliis oppositis petiolatis peltatis coriaceis late ovatis apice abrupte et brevissime caudato-acuminatis basi rotundatis 35 cm. longis 20 cm. latis supra subtusque glabratis; petiolo 12 cm. longo glabrato; inflorescentiis plus minusve fasciculatis subumbellatis 4–5-floris; pedunculo minute ferrugineo-tomentello petiolo multo breviore; pedicellis 1 cm. longis ut in pedunculo vestitis; bracteis oblongo-lanceolatis acuminatis pedicellis subaequantibus; calycis lobis foliaceis oblongis abrupte mucronulatis 1.25–1.5 cm. longis extus glabratis inferne sparsissime tomentellis; corollae speciosae ut videtur flavidulae extus omnino glabrae tubo-proprio late cylindrico 2 cm. longo basi ca. 0.5 cm. diametro metiente ad apicem staminigero ibique gradatim dilatato faucibus late tubulatis 2.5 cm. longis ostio ca. 1 cm. diametro metiente lobis late obovatis dolabriformibus 2 cm. longis patulis; antheris anguste sagittatis 1.25 cm. longis; ovariis late ovoideis 0.25 cm. longis dense ferrugineo-tomentellis; stigmate 0.2 cm. longo; nectariis ovoideo-dentiformibus ovariis vix aequantibus; folliculis ignotis.—BOLIVIA: exact locality and date lacking, *M. Bang 2804* (Mo. Bot. Garden Herbarium, TYPE).

At present the only species of *Peltastes* with a tubular corolla-throat by which it may readily be distinguished as well as by means of the unusually large leaves.

Peltastes colombianus Woodson, sp. nov., suffruticosa volubilis; ramis teretibus crassiusculis juventate minute ferrugineo-puberulis mox glabratis; foliis oppositis longe petiolatis peltatis rigide membranaceis ovatis vel late ovalibus apice abrupte brevissimeque caudato-acuminatis basi rotundatis 18–25 cm. longis 15–20 cm. latis supra minutissime papillatis in umbilico plus minusve ferrugineo-tomentulosis subtus glabris; petiolis 8–10 cm.

longis, juventate minute ferrugineo-tomentellis tandem glabratis; inflorescentiis paniculatis cymosis; pedunculis petiolo paulo brevioribus 10–15-floris minute ferrugineo-tomentellis; pedicellis 1.5–2.0 cm. longis; bracteis oblongo-spathulatis petiolo paulo brevioribus; calycis laciniis late oblongo-spathulatis breviter acuminatis 2.5–3.0 cm. longis basi sparse tomentellis superne glabratis intus multiglandulosis; corollae magnae extus glabrae tubo-proprio latiuscule cylindrico 2.5–2.75 cm. longo basi ca. 0.4 cm. diametro metiente faucibus gradatim dilatatis conicis 1.5 cm. longis ostio ca. 1.5 cm. diametro metiente lobis obovato-orbiculatis ca. 1 cm. longis patulis; antheris anguste sagittatis 1.25 cm. longis; ovariis ovoideis ca. 0.2 cm. longis minute ferrugineo-tomentellis; nectariis ovoideo-dentiformibus ovariis paulo brevioribus; folliculis ignotis.—COLOMBIA: vicinity of Santa Marta, alt. 2000 ft., June, 1899, *H. H. Smith 2412* (Mo. Bot. Garden Herbarium, TYPE).

Approaches *P. macrocalyx*, but differs conspicuously in its much larger, nearly glabrous foliage, its broader calyx-lobes, and its gradually dilating, conical corolla-throat. The latter species, moreover, is apparently confined to southeastern Brazil.

Of the six species referred to the genus *Peltastes* in the paragraphs immediately preceding, *P. peltatus* and *P. macrocalyx* were recognized by Mueller-Argoviensis as belonging to the inclusive genus *Echites*, although he noted in them certain characters in common with his monotypic genus *Stipecoma*, such as the peltate leaves and rostrate seeds. Miers, in 1878, was quick to take advantage of Mueller's observations, and transferred the species mentioned unequivocally to *Stipecoma*.

Although sharing with *Stipecoma* such conspicuous features as peltate leaves, rostrate seeds, and a somewhat similar geographical distribution, *Peltastes* differs in its opposite, lateral inflorescences, infundibuliform corolla, foliaceous bracts and calyx-lobes, exceedingly numerous and indefinitely distributed squamellae, and more robust vegetative habit. The distinction in vegetative habit is further accentuated in a very tangible way by the possession of a conspicuous, ferruginous indument by the six known species of *Peltastes*, since the single species of *Stipecoma* is glabrous in all parts.

Peltastes differs from *Echites* P. Br. in its opposite, lateral inflorescences, infundibuliform corolla, indefinitely distributed squamellae, and peltate leaves. Technical details of the reproductive organs also differ.

Fernaldia brachypharynx Woodson, sp. nov., suffruticosa volubilis; ramis teretibus minute puberulis tandem glabratis; foliis oppositis longiuscule petiolatis membranaceis late ovatis apice subiter acuminatis basi rotundatis 7–10 cm. longis 5–7 cm. latis supra sparse minutissimeque pilosulo-papillatis subtus pallidioribus minute sparseque puberulis; petiolis 1.5–2.0 cm. longis sparse pilosulis; inflorescentiis pseudo-racemosis multifloris; pedunculo minute puberulo petiolo paulo superante; pedicellis prope apicem pedunculi congestis ca. 0.5 cm. longis post maturitatem paulo accrescentibus; bracteis minute ovato-lanceolatis scariaceis 0.1–0.2 cm. longis; calycis laciniis ovatis acuminatis 0.2–0.3 cm. longis extus sparse pilosulis intus basi squama deltoidea margine minute crenulata instructis; corollae speciosae ut videtur albidae extus in alabastro omnino pilosulae tubo-proprio latiuscule cylindrico 1.75–2.0 cm. longo basi ca. 0.3 cm. diametro metiente faucibus anguste conicis 1.5–1.75 cm. longis ostio ca. 0.75 cm. diametro metiente lobis oblique obovatis acuminatis ca. 1.25 cm. longis patulis; antheris oblongo-sagittatis basi obtuse auriculatis 0.6 cm. longis; ovariis oblongoideis ca. 0.15 cm. longis glabris; stigmate fusiformi apice longe-apiculato ca. 0.2 cm. longo; nectario ut in *F. pandurata* lobato ovariis ca. dimidio aequante; folliculis desiderantur.—GUATEMALA: along the road from Escuintla to the port of San José de Guatemala, Aug. 23, 1860, *S. Hayes s. n.* (Gray Herb., TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

This striking species may easily be distinguished from *F. pandurata* (A. DC.) Woodson by means of its narrowly conical corolla-throat which about equals in length the broadly cylindrical proper-tube, contrasting sharply with the broadly campanulate throat and much longer, narrower proper-tube of the latter species. The flower-buds of *F. brachypharynx*, moreover, are generally pilosulose without, while those of *F. pandurata* are merely somewhat ciliolate at the tip.

Thenardia tubulifera Woodson, sp. nov., suffruticosa volubilis; ramulis teretibus tenuibus glabris; foliis oppositis breviter petiolatis membranaceis elliptico-lanceolatis apice subcaudato-acuminatis basi obtusis 4–7 cm. longis 1.5–2.5 cm. latis supra glabris subtus in nervo medio venisque laxe pilosulis; petiolis 0.5–1.0 cm. longis minutissime puberulis; inflorescentiis subumbellato-corymbosis pedunculo 2.5–3.0 cm. longo ad apicem trichotomo ramulis 0.5–0.75 cm. longis; pedicellis prope apices ramulorum congestis ca. 2.5 cm. longis post maturitatem paulo accrescentibus glabris; bracteis lanceolatis minutis; calycis laciniis ovatis acutis 0.2 cm. longis extus glabris intus squama deltiforma subintegra ca. 0.75 cm. longa instructis; corollae salverformis haud rotatae tubo late cylindrico prope medium paulo constricto 0.5–0.6 cm. longo basi ca. 0.2 cm. diametro metiente; lobis obovato-orbicularibus breviter oblique apiculatis 0.75 cm. longis patulis; antheris anguste sagittatis 0.7–0.8 cm. longis omnino exsertis; ovariis ovoideis 0.15 cm. longis glabris; stigmate fusiforme apice breviter bipartito ca. 0.125 cm. longo; nectariis late ovoideis integris ovariis vix aequantibus; folliculis ignotis.—MEXICO: Jalisco: exact locality and date lacking, *L. Diquet s. n.* (N. Y. Bot. Garden Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

Of the general aspect of *Th. floribunda* HBK., but differing from the other known species of the genus in the development of a conspicuous corolla-tube. *Th. tubulifera* may be distinguished from the latter species also in the pubescence of the lower surface of the leaves which occurs generally along the midrib and veins and not merely in small patches in the axils of the midrib.

Echites turrigera Woodson, sp. nov., suffruticosa volubilis; ramulis teretibus gracilibus juventate minute sparseque pilosulis mox glabratis; foliis oppositis longiuscule petiolatis rigidiuscule membranaceis ovato-oblongis apice acuminatis basi obtusis plerisque 3.5–5.0 cm. longis 2.25–3.0 cm. latis maturitate omnino glabris dilute viridibus opacis; petiolis 0.75–1.0 cm. longis; inflorescentiis cymosis lateralibus alternatis 14–20-floris; pedunculo foliis subaequante sparse pilosulo; pedicellis 1.0 cm. longis post maturitatem paulo accrescentibus ut in pedunculo vestitis;

bracteis linearibus 0.2–0.5 cm. longis scariaceis vel parum subfoliaceis; calycis laciniis anguste lanceolatis acuminatis 0.75 cm. longis subfoliaceis extus glabriusculis intus basi squamellas 4–6 dentiformes gerentibus; corollae salverformis colore dilute flavoviridis tubo gracillimo 2.75 cm. longo basi cylindrico ca. 0.2 cm. diametro metiente paulo infra medium conspicue dilatato ibique staminigero deinde faucem versus sensim attenuato lobis oblique obovatis acuminatis 0.75 cm. longis patulis; antheris anguste sagittatis basi acutissime auriculatis 0.75 cm. longis; ovariis oblongoideis 0.3 cm. longis glabris; nectariis ovoideis compressis ovariis dimidio aequantibus; folliculis ignotis.—GUATEMALA: Gualan, alt. 620 ft., June 20, 1909. *C. C. Deam 6376* (Mo. Bot. Garden Herbarium, TYPE).

The nearest ally of the foregoing is apparently *E. yucatanensis* Millsp., from which it differs in the larger, paler leaves which are not pandurate, the longer, subfoliaceous calyx-lobes, and the more floriferous, more perfectly cymose inflorescence. The specific adjective is in fanciful allusion to the clustered, spire-like floral buds.

Galactophora magnifica Woodson, sp. nov., suffrutescens omnino glabra; caulibus erectis teretibus sat crassis altitudine ignotis; foliis oppositis sessilibus coriaceis late ovato-cordatis apice obtusis basi amplexicaulibus 4.5–5.0 cm. longis 3.0–3.5 cm. latis subtus pallidis margine in sicco subrevolutis; inflorescentiis terminalibus ca. trifloris; pedunculo quam folio multo breviore; pedicellis 1 cm. longis; bracteis minutis vix bene visis; calycis laciniis ovato-lanceolatis acuminatis 2.0–2.5 cm. longis 0.75–1.0 cm. latis scariaceis vel parum petaloideis extus glabris intus basi squamellas minimas extra-axillares gerentibus; corollae speciosissimae infundibuliformis (colore aut roseae aut dilute flavidulae?) tubo-proprio breviuscule cylindrico 2.0-2.25 cm. longo basi ca. 0.4 cm. diametro metiente ad apicem staminigero ibique dilatato faucibus late campanulatis 3 cm. longis ca. 2.5 cm. diametro metiente lobis late ovatis breviter acuminatis 4.5 cm. longis patulis patentibusve; antheris anguste lanceolatis 0.8 cm. longis basi acute biauriculatis dorso glabriusculis; nectario cupuliformi plus minusve crenulato ca. 0.1 cm. alto ovariis oblongoideis gla-

bris ca. ⅛ aequante; folliculis ignotis.—BRAZIL: Procedencia Juruena, "campo humido e pantuoso," April, 1909. *F. C. Hoehne 1759* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

This species is probably the most striking of the genus *Galactophora* known at present. Like *G. calycina* (Hub.) Woodson, it is without the peculiar aculeolate glands which characterize the exterior of the corolla, calyx, and stems of the other species, but it differs from the latter in the large, ovate leaves, and particularly in the much larger corolla with showy, spreading lobes.

Temnadenia ornata (Hoehne) Woodson, comb. nov.

Echites ornata Hoehne, Comm. Linh. Telegr. Estrat. Matto Grosso, Annexo 5, Bot. **6**: 82. *pls. 120, 131, fig. 1.* 1915.

The genus *Temnadenia*, as established by Miers, Apoc. So. Am. 207, 1878, is an extremely unnatural conglomeration of twenty-two species of widely separate affinities. Of those originally transferred to the genus, one species is more correctly included within the genus *Tabernaemontana* L., one within *Dipladenia* A. DC., eight within *Mandevilla* Lindl., and eight within *Prestonia* R. Br. The remaining four constitute a fairly natural entity to which Miers's generic name must be applied. *Temnadenia*, as thus restricted, is found to be a genus very closely related to *Echites* P. Br., differing chiefly in its di- or trichotomous, indeterminate inflorescence and structural details of the reproductive organs. The flowers of *Echites* are always pale greenish-yellow, while those of *Temnadenia* are a rich cream suffused with pink, or in one species, *T. violacea* (Vell.) Miers, a nearly uniform, rich crimson gradually paling toward the base of the corolla-tube. The geographical distribution of the two genera also differs, that of the former being limited to the Greater Antilles, the Bahama Islands, southern Florida, Yucatan, and Guatemala, and that of the latter to southern Brazil.

Mandevilla sertuligera Woodson, sp. nov., suffruticosa volubilis; ramulis gracillimis teretibus juventate minute hispidulis puberulisve tandem glabratis maturitate rubidulis parum rimosis; foliis oppositis petiolatis membranaceis elliptico-ovatis acumi-

natis obscure cordatis plerisque 6–8 cm. longis 3.0–3.5 cm. latis supra hispidulo-strigillosis subtus dense lanato-tomentulosis; petiolis 0.75–1.25 cm. longis ut in ramulis vestitis; squamis stipulaceis haud visis; inflorescentiis racemosis corymbosis lateralibus alternatis vel subterminalibus multifloris; pedunculo foliis ca. dimidio breviore hispidulo; pedicellis congestis subsecundis 0.3–0.4 cm. longis post maturitatem paulo accrescentibus; bracteis linearibus pedicellis subaequantibus scariaceis; calycis lobis anguste lanceolatis longe acuminatis subsetaceis 0.4–0.6 cm. longis scariaceis extus pilosulis intus basi squamellas multas denticuliformes uniforme gerentibus; corollae tubiformis dilute flavidulae tubo cylindrico 0.75 cm. longo basi ca. 0.2 cm. diametro metiente infra medium staminigero faucibus paulo ampliatis lobis late ovatis acutiusculis erectis 0.3–0.4 cm. longis; antheris anguste oblongis 0.4 cm. longis basi obscure auriculatis; ovariis ovoideis minute puberulo-papillatis in stylo abrupte contractis 0.15 cm. longis; nectarii glandulis oblongo-ovoideis basi connatis ovariis paulo superantibus; folliculis ignotis.—MEXICO: Michoacan: rocky hills near Coru Station, alt. 6000 ft., Jan. 23, 1907. *C. G. Pringle 13890* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

Most closely related to *M. Syrinx* Woodson, from which it differs in its shortly pedunculate, corymbose inflorescence and extremely long-attenuate or subsetaceous calyx-lobes which are nearly twice as long as in the latter species. In addition to the type specimen, the species is represented in several of the leading herbaria of America and Europe by two other collections by Pringle in the Mexican states of Michoacan and Morelos.

Mandevilla rugosa (Benth.) Woodson, comb. nov.

Echites rugosa Benth. in Hook. Journ. Bot. 3: 249. 1841.

Amblyanthera versicolor (Stadelm.) Muell.-Arg. *β. intermedia* Muell.-Arg. in Mart. Fl. Bras. 6[1]: 146. 1860, in part.

Mitozus rugosus (Benth.) Miers, Apoc. So. Am. 222. 1878.

This member of the puzzling *mollissima-scabra* complex differs from the latter species chiefly in the coriaceous or subcoriaceous, strikingly rugose foliage.

Mandevilla rutila Woodson, sp. nov., suffruticosa volubilis; ramulis gracillimis juventate ferrugineo-pilosulis tandem glabratis; foliis oppositis breviter petiolatis membranaceis late ellipticis acuminatis obscure auriculatis 8–15 cm. longis 3–5 cm. latis supra minute ferrugineo-pilosulis nervo medio parce glanduligeris subtus dilute ferrugineo-pilosulis; petiolis 0.5–1.0 cm. longis sparse pilosulis; squamis stipulaceis obsoletis vel minus manifestis; racemis simplicibus lateralibus alternatis pedunculo foliis saepissime aequale multifloro; pedicellis 0.3–0.5 cm. longis maturitate parum accrescentibus; bracteis linearibus filiformibusve ca. 1 cm. longis scariaceis; calycis laciniis ovato-lanceolatis longeacuminatis 0.2–0.4 cm. longis scariaceis basi intus squamellam deltatam oppositam gerentibus; corollae infundibuliformis fulvoaurantiacae extus sparse pilosulae tubo-proprio anguste cylindrico superne parum gibboso 1.75–2.25 cm. longo basi ca. 0.15 cm. diametro metiente faucibus conicis 1.0–1.5 cm. longis ostio ca. 0.75–1.0 cm. diametro metiente lobis oblique obovatis acuminatis 1.25–1.75 cm. longis patulis; antheris anguste ellipticis obscure auriculatis 0.4 cm. longis; ovariis ovoideis in stylo gracili gradatim productis 0.15 cm. longis glabris; stigmate 0.2 cm. longo breviter apiculato; nectariis 5 oblongoideis ovariis subaequantibus; folliculis gracillimis conspicue articulatis 15–20 cm. longis glabris; seminibus 0.5 cm. longis como aurantiaco ca. 2 cm. longo.—BOLIVIA: La Paz: Mapiri, alt. 5000 ft., April, 1886. *H. H. Rusby 2385* (N. Y. Bot. Garden Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

Most closely related to *M. scabra* (R. & S.) K. Sch., from which it may be distinguished by means of its much longer, linear to filiform bracts, pale-ferruginous foliar indument, and longer, more attenuate calyx-lobes. At present five different collections of *M. rutila* are known, all from the province of La Paz, Bolivia. *M. scabra* is apparently confined to Venezuela, the Guianas, and northern Brazil.

Secondatia Macnabii (Urb.) Woodson, comb. nov.

Orthechites Macnabii Urb. Symb. Ant. 6: 37. 1909.

The monotypic genus *Orthechites* is distinguishable from *Secondatia* A. DC. merely by a slight constriction of the corolla-

tube at the insertion of the stamens, and by lanceolate calyx-lobes. In all other essential respects, the two genera are quite conformable and surely appear to constitute a natural unity. No one as yet has proposed the establishment of a segregate genus upon the basis of the glabrous anthers of *S. peruviana* Poeppig or the lateral cymes of *S. Schlimiana* Muell.-Arg. and *S. floribunda* A. DC., and the distinguishing characteristics of *S. Macnabii* likewise appear to be merely specific, or at most sectional, in nature. Since *S. Macnabii* is apparently confined to Jamaica, the genus *Secondatia* is found to have a type of geographical distribution somewhat similar to that of *Mandevilla* Lindl. subgen. *Eumandevilla*.

Odontadenia laxiflora (Rusby) Woodson, comb. nov.

Laubertia (?) *laxiflora* Rusby, Bull. N. Y. Bot. Gard. **4**: 408. 1907.

A species superficially differing from others of *Odontadenia* Benth. in the somewhat smaller flowers, but evidently congeneric in all other essential particulars. The characters of *Odontadenia* must be rather liberally interpreted unless such segregate genera as *Anisolobus* A. DC. and *Perictenia* Miers are to be regarded as valid. The distinguishing characters of the little-understood genus *Laubertia* A. DC. have been briefly discussed in a previous note of this series (Ann. Mo. Bot. Gard. **18**: 556. 1931).

Mesechites minima (Britton & Wilson) Woodson, comb. nov.

Echites minima Britton & Wilson, Mem. Torrey Bot. Club **16**: 94. 1920.

A most appropriately named species indigenous to central and southern Cuba, a range shared by its only Cuban congener, the familiar, showy-flowered *M. myrtifolia* (R. & S.) Muell.-Arg. (= *Echites rosea* A. DC.).

In restoring at this time the genus *Mesechites* Muell.-Arg. which has been in disuse since 1878, only a few words are necessary in justification. From *Echites* P. Br. (in the stricter sense as typified by *E. umbellata* Jacq.), with which it is still confused, *Mesechites* may readily be distinguished by its glandular foliage, dichotomous, bostrychoidally racemose inflorescence, obscurely

auriculate anthers, and multiglandular calyx. Although persistently confounded with *Echites*, *Mesechites* is more naturally to be associated with *Mandevilla* Lindl., from which it is quite easily separable because of its dichotomous bostrychoid inflorescence and fusiform stigma. The foliar glands of most species of *Mesechites* also are quite distinctive, usually being more or less laminate in form and clustered concentrically at the very base of the midrib, conditions never observed among species of *Mandevilla*.

Mesechites bicorniculata (Rusby) Woodson, comb. nov.

Echites bicorniculata Rusby, Descr. So. Am. Pl. 86. 1920.

Differing from the closely related *M. trifida* (Jacq.) Muell.-Arg. chiefly in the possession of a conspicuous vegetative indument.

Mesechites Sanctae-Crucis (S. Moore) Woodson, comb. nov.

Echites Sanctae-Crucis S. Moore, Trans. Linn. Soc. Bot. III. **4**: 396. 1895.

Echites trifida Jacq. var. *Sanctae-Crucis* (S. Moore) Malme, Bull. Herb. Boiss. II. **4**: 196. 1904.

Apparently deserving of specific rank because of its shorter corolla-lobes, exappendiculate nodes, and comparatively restricted, more southerly distribution (Paraguay, adjacent Bolivia, Argentina, and Brazil), by which it may be distinguished from *M. trifida* (Jacq.) Muell.-Arg.

Mesechites citrifolia (HBK.) Woodson, comb. nov.

Echites citrifolia HBK. Nov. Gen. **3**: 216. 1818.

Echites brevipes Benth. Pl. Hartw. 216. 1849.

Mesechites brevipes (Benth.) Muell.-Arg. Linnaea **30**: 454. 1860.

Mitozus brevipes (Benth.) Miers, Apoc. So. Am. 223. 1878.

A comparison of the type specimen of *E. citrifolia* (*Humboldt & Bonpland s. n.* in Hb. Mus. Hist. Nat. Paris) with that of *E. brevipes* (*Hartweg 1195* in Hb. Brit. Mus.) permits no doubt concerning the necessity of this combination. Photographs of either specimen are deposited in the herbarium of the Missouri Botanical Garden.

SOME NEW SPERMATOPHYTES FROM TEXAS[1]

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In a collection of plants made by Mr. John A. Moore and the writer from the mountains of western Texas during the summer of 1931 several new species and one new variety were discovered.

Cladothrix lanuginosa Nutt. var. **carnosa** Steyermark, n. var.

Caulibus usque ad ramulos floriferos glabrescentibus; foliis caulinis involucralibusque parvis, 4–13 mm. longis, 3–9 mm. latis, carnosis, caulinis omnibusque praeter involucralibus supremis glabrescentibus. (Gravelly flat, 3 mi. east of Study Butte, Brewster Co., Texas, alt. 762 m., June 26, 1931, *Moore & Steyermark 3795*.—TYPE, in Gray Herb.).

Stems, up to the floriferous branchlets, glabrescent; cauline and involucral leaves small, 4–13 mm. long, 3–9 mm. broad, fleshy, the cauline and all except the uppermost involucral leaves glabrescent.

This variety is distinguished from *Cladothrix lanuginosa* by its stem being glabrescent up to the floriferous branches, instead of pubescent throughout, by its fleshy, instead of soft membranaceous, cauline and involucral leaves, and by its cauline and all except the uppermost involucral leaves being glabrescent instead of stellate-pubescent. The uppermost involucral leaves in the species are densely and conspicuously stellate-pubescent, producing a white cottony appearance at the summit of the inflorescence, whereas in the variety the pubescence is inconspicuous and does not produce the cottony effect. The leaves of the variety are on the whole smaller than those of the species.

The variety was found growing in abundance in an arid gypsum flat several miles north of the Rio Grande in soil heavily impregnated with alkaline salts, in association with such calciphiles as *Atriplex canescens*, *A. acanthocarpa*, *Suaeda suffrutescens*, *Greggia camporum*, and *Nama Havardii*. The extremely alkaline soil here has probably been a leading factor in producing the fleshy-leaved condition.

[1] Issued November 15, 1932.

To this variety should be referred Havard's no. 104 collected around Tornillo Creek, western Texas (Gray Herb.), a locality about thirty miles east of the one from which the present collection was made.

Polygala rimulicola Steyermark, n. sp.

Perennis tenuis e basi lignosa; caulibus multis, 1–5 cm. longis, ramosis, prostratis vel parum ascendentibus, gracillimis, confertim foliosis, viridibus, glabris sub lente papillis plurimis minutis et pilis brevissimis, mollibus sparsis incurvatis, obsitis; foliis omnino similibus, parum carnosis, glabris sub lente minute papillosis sparseque puberulentibus, elliptico-ovatis, acutis vel parum cuspidatis, subsessilibus, 1.5–4 mm. longis, 1–2.5 mm. latis; floribus 1–2, terminalibus, 5 mm. longis; pedicellis maturitate recurvatis nutantibusque, ca. 2 mm. longis; sepalo superiore ovato, acuto, glabro, persistente, roseo-purpurascenti alboque, 1.5–2 mm. longo, 1 mm. lato; sepalis inferioribus libris, oblongo-obovatis, acutis, deciduis, glabris, roseo-purpurascentibus albisque, 2–3 mm. longis, 1 mm. latis; alis roseo-purpurascentibus albisque, deciduis, obliquis, late obovatis, apice obtusis, basi angustatis, venosis, glabris, 4 mm. longis, 2.5 mm. latis; carina ca. 3 mm. longa, subviridi-flava, e rostrata, inflata, saccata, apice suboblique truncata angulis rotundatis basi superiore, appendicibus duobus brevibus parallelis rectis ornata, ad basim floris adversis, tertia parte superiore pilis plurimis brevibus erectis tenuibus tecta; petalis superioribus basi albis, apice purpureis, longitudine ⅜ carinam adnatis, lineari-oblongis, apice subtruncato, intus pilis paucis brevibus tecto, 4.5 mm. longis, ca. 1 mm. latis, staminibus 7; capsula late obovato-ovata, emarginata, venosa, parce pilis brevibus incurvatis tecta, dense pilosa in sino apicis emarginatae, ca. 2 mm. longa, 1.5–2 mm. lata; semine oblongo, dense sericeo-pubescente pilis longis, 1–1.5 mm. longo; arillo 0.5 mm. longo, glabro, corneo, globoso, latere utraque umbone magno ornato. (Exposed rock crevices, Smith Canyon, Guadalupe Mts., Culberson Co., Texas, alt. 1900 m., July 20, 1931, *Moore & Steyermark 3515*.—TYPE, in Gray Herb.).

A delicate perennial from a woody base; stems numerous, very slender, prostrate or slightly ascending, branching, 1–5 cm. long,

green, apparently glabrous but under high magnification covered with numerous minute papillae and short soft scattered incurved hairs; leaves similar throughout, subsessile, elliptic-ovate, 1.5–4 mm. long, 1–2.5 mm. broad, acute to slightly cuspidate, slightly fleshy, apparently glabrous, but under high magnification covered with numerous minute papillae and few short soft scattered hairs; flowers 1–2, terminal, 5 mm. long; pedicels at maturity recurved and nodding, 2 mm. long; upper sepal persistent, rose-purplish and white, ovate, 1.5–2 mm. long, 1 mm. broad, acute, glabrous; lower sepals free, deciduous, rose-purplish and white, oblong-obovate, 2–3 mm. long, 1 mm. broad, acute, glabrous; wings deciduous, rose-purplish and white, oblique, broadly obovate, 4 mm. long, 2.5 mm. broad, obtuse at apex, narrowed at base, veiny (the veins ending free), glabrous; keel unbeaked, inflated, greenish-yellow, pouch-shaped, 3 mm. long, the outer upper and lower ends rounded, the inner upper end terminating in 2 short parallel straight appendages extending towards the base of the flower, the upper third of the keel covered with numerous erect short fine hairs; upper petals united to the keel about ⅜ their length, white at the base, purplish-red at the apex, linear-oblong, 4.5 mm. long, 1 mm. broad, subtruncate at the apex, slightly puberulent within; stamens 7; capsule venose, broadly obovate-oval, 2 mm. long, 1.5–2 mm. broad, emarginate, sparingly covered with short incurved hairs, densely pubescent in the sinus of the emarginate apex; seed oblong, 1–1.5 mm. long, densely sericeous-pubescent with long hairs; aril globose, 0.5 mm. high, with a large umbo at each side, corneous, glabrous.

A well-marked species occurring in crevices of shaded to slightly exposed limestone boulders or cliff-faces in moist ravines of the Guadalupe Mountains.

The combination of characters distinguishing this species are the glabrous globose aril with no conspicuous lobes, the unbeaked keel, persistent upper sepal, prostrate habit, minute leaves and flowers, and essentially glabrous condition of stem, leaves, and fruit.

In habit this species slightly approaches *Polygala macradenia*, but the latter has a pubescent umbo, a 3-plicated beaked keel, thickly gland-dotted leaves, sepals, and fruit, and erect to ascending, instead of prostrate, stems.

It is related to *Polygala acanthoclada*, which it resembles in the beakless keel, and to *P. eucosma*, to which it is more closely allied. The Mexican *P. Purpusii*, *P. Conzattii*, and *P. Parryi*, with their glabrous arils, persistent upper sepals, unbeaked keels, and deciduous lower sepals, also are closely allied to this species, but, as Dr. S. F. Blake observes, the seed of *P. rimulicola* is unique in its group.

Laphamia quinqueflora Steyermark, n. sp.

Perennis humilis multum ramosus effuse patulus e caudice robusto lignoso, caespitem confertum formans; caulibus erectis, divaricate corymbifereque ramosis, teretibus, striatis, minute puberulentibus, pullo-viridibus, 1–1.4 dm. altis; petiolis 0.8–1.3 cm. longis, minute puberulentibus; laminis late subrotundato-ovatis aut suborbiculatis, pullo-olivaceo-viridibus, nitidis, membranaceis, oppositis, integris vel leviter repandis, glabris vel pilis paucis sparsis minutis obsitis, obtusis, apice rotundatis, 0.5–1.5 cm. longis, 0.5–2 cm. latis; capitulis eligulatis, multis, 7 mm. longis, ca. 4 mm. latis, plerumque ca. quinque floribus, terminalibus, solitariis, corymbose dispositis; involucri bracteis 5, 2-seriatis, aequalibus, lineari-oblongis, obtusis, carinatis, carina convexa, apice leviter incurvato, 6–7 mm. longis, 1–1.5 mm. latis, moderatim minuteque puberulentibus; disci corollis 4.5–5 mm. longis, limbo cylindraceo subito in tubulum angustum angustato, limbo ca. duplo longiore quam tubulo, minute puberulente; pappo 22–26 aristis inaequalibus setosis barbellulatis, 12–14 brevioribus 10–12 longioribus composito, aristis longissimis, ca. 2 mm. longis, corollae tubulum angustatum, vix excedentibus; achaenio compresso, oblongo-cuneato, basi, paullo angustato, apice truncato, stramineo vel fulvo, hirtello, faciebus medio 1-nervatis. (Niches in exposed limestone cliffs, Lower McKittrick Canyon, Guadalupe Mts., Culberson Co., Texas, alt. 1900 m., July 20, 1931, *Moore & Steyermark 3547*.—TYPE, in Gray Herb.).

A low much-branched, diffusely spreading perennial from a stout woody caudex, forming a dense mat, 1–1.4 dm. high; stems numerous, slender, erect, terete, divergently and corymbosely branched, striate, minutely puberulent, dark green; petioles minutely puberulent, 0.8–1.3 cm. long; blades opposite, broadly

subrotund-ovate or suborbicular, 0.5–1.5 cm. long, 0.5–2 cm. broad, entire or slightly repand, obtuse and rounded at apex, membranaceous, essentially glabrous, dark olive-green, lustrous; heads discoid, numerous, terminal, corymbosely clustered, mostly 5-flowered, 7 mm. long, 4 mm. broad; involucral bracts 5, in 2 series, equal, linear-oblong, 6–7 mm. long, 1–1.5 mm. broad, obtusish, carinate with a convex keel, apex slightly incurved, margins subscarious, convex and slightly incurved, moderately and minutely puberulent; disk-corollas 4.5–5 mm. long, the cylindrical throat abruptly contracted into a narrow tube, the throat about 1¾ times as long as the tube, minutely puberulent; pappus of 22–26 unequal setose barbellate awns, consisting of 10–12 medium to long awns associated with 12–14 shorter ones, the longest awns 2 mm. long, slightly surpassing the constricted corolla-tube; achene compressed, oblong-cuneate, truncate at apex, hirtellous, stramineous to light brown, the faces 1-nerved in the middle.

A very distinct and well-marked species, differing from any of the other species in the genus. In having a pappus of 20 or more unequal rigid hispidulous bristles and a corolla with short proper tube and cylindraceous throat, it properly belongs in the section *Pappothrix*, but differs from the other species in that section especially in having the heads usually 5-flowered. Its distinctive characters are the 5-flowered heads, each of 5 involucral bracts, the essentially glabrous, membranaceous, subrotund, mostly entire or slightly repand leaves, and the pappus of 22 to 26 unequal setose hispidulous bristles.

This species was found growing on vertical exposed moist faces of limestone cliffs in deep canyons at an elevation of 1900–2590 meters (6000–8500 ft.), where it was conspicuous in dense hemispherical clumps, with its lustrous, dark olive-green leaves.

Valeriana texana Steyermark, n. sp.

Perennis caudice denso rugoso multum ramoso; caulibus pluribus, erectis, 1–1.5 dm. altis, gracilibus, fere glabris; foliis radicalibus multis, oblanceolatis vel obovatis, in petiolum longum gracilem paullo alatum contractis, petiolis inclusis, 4–10 cm. longis, 0.6–1.8 cm. latis, glabris, obtusis, integris, longitudine

usque ad ⅔ altitudinem caulis attingentibus; foliis caulinis 1–2 jugis, omnibus simplicibus, oblanceolatis, plerumque brevioribus quam radicalibus, 1–4.5 cm. longis, lobis lateralibus nullis, integris; inflorescentia cyma thyrsiformi composita, multiflora, anthesi valde contracta, pedunculis superioribus oppositis, inferioribus alternatis; floribus parvis, subflavo-albis, multis; corolla 5.5 mm. longa, cum tubo infundibuliformi; staminibus 3, inclusis; fructu oblongo, in apicem truncatum paullo angustato, 3–3.5 mm. longo., prope 1.5 mm. lato, glabro. (On boulders in creek, Upper McKittrick Canyon, Guadalupe Mts., Culberson Co., Texas, alt. 2000 m., July 21, 1931, *Moore & Steyermark 3528.*—TYPE, in Gray Herb.).

Perennial from a thick rugose much-branched ligneous rootstock, 1–1.5 dm. high; stems several, slender, erect, with 1–2 pairs of cauline leaves, essentially glabrous; basal leaves numerous, contracted into a long slender slightly margined petiole, ⅔ the height of the stems, 4–10 cm. long, 0.6–1.8 cm. broad, oblanceolate to obovate, entire, obtusish, glabrous, light green; cauline leaves in 1–2 pairs, all simple, lateral lobes absent, oblanceolate, much shorter than the radical ones, 1–4.5 cm. long, entire; inflorescence a compound thyrsiform cyme, numerously flowered, much contracted at anthesis, the upper peduncles opposite, the lower alternate; flowers small, very numerous, yellowish-white; corolla 5.5 mm. long, the infundibuliform tube 3 times as long as the broadly ovate lobes; stamens 3, included; fruit compressed, oblong, 3–3.5 mm. long, 1.5 mm. broad, slightly narrowed towards the truncate apex, glabrous.

This is the first collection of a species of *Valeriana* from Texas, so far as the writer is aware. It was found on moist shaded limestone cliffs in the ravines of various canyons in the Guadalupe Mountains of Texas, growing at an altitude of 1828–2438 meters (6000–8000 ft.).

It differs strikingly from other North American species of this genus, especially in having the leaves simple and undivided throughout, in the inflorescence much contracted into a compound thyrsiform cyme the length of which averages about one-half that of the flowering stem, and in a strongly developed multicipital caudex. Only *Valeriana pubicarpa* and *V. wyomingensis*

approach this new species. From the former it may be distinguished by the thyrsiform, instead of corymbiform or subcapitate, inflorescence, by the glabrous stems and fruit, and by the simple cauline leaves throughout. From the latter it differs in its strongly developed ligneous caudex, many-flowered contracted inflorescence, instead of a few-flowered, loose and open type, and in its longer corolla which is infundibuliform instead of campanulate.

COCCIDIOIDAL GRANULOMA: A CLASSIFICATION OF THE CAUSATIVE AGENT, COCCIDIOIDES IMMITIS[1]

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HISTORY

The purpose of this paper, as the title indicates, is to attempt a proper determination in the classification of fungi of the agent responsible for the disease clinically known by various names, as "blastomycosis" (Montgomery and Ormsby, '08), "oidiomycosis" (Giltner, '18, Ricketts, '01), "protozoic dermatitis" (Montgomery, '00), "dermatitis coccidioides" (Montgomery, Ryfkogel and Morrow, '03, Wolbach, '04), "blastomycetic dermatitis," "coccidioidal granuloma," and the "California disease." Of this list, coccidioidal granuloma has the most widespread acceptance, and in all references in this paper to the pathologic, clinical, or other diagnostic features of the fungus, this term will be used.

Since the report of the first case by Wernicke ('92) there have been 286 cases recorded, and with the one involved in this paper the total now is 287. The disease has received a great deal of attention because of its mode of infection, complicated diagnosis, lack of definitely demonstrable prophylactic measures, great percentage of fatality, peculiar pathogenic abilities, and last, but not least, the indefinite classification of the fungus itself.

In order to present a clearer perspective of the field, a résumé of the noteworthy points involved would be in order.

As stated above, the first case was reported by Wernicke ('92) from Buenos Aires. Clinically, the case was diagnosed as "mycosis fungoides." During the examination spherical organisms resembling protozoa were found in the lesions, and the cause of the disease was considered due to these bodies. Later reports showed that the patient had died of a general infection.

The second case was reported by Rixford in a brief note in the 'Occidental Medical Times' of 1894, later published with Gilchrist in 'Johns Hopkins Hosp. Repts.' ('96). Six months later in the same year, in conjunction with Thorne, he reported another

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case in the latter journal. In these two cases, the round organisms were seen in the smears made from the lesions, especially the purulent exudate. Various stages in the development were observed, some smears being filled with difficultly stained, irregular masses of material and some with a highly refractile, clear, non-staining capsule surrounding a group of spores formed endogenously, and stages between the two.

Pathologically, the condition was perplexing inasmuch as it simulated tuberculosis in all its clinical entities. Histological sections showed giant cell formation in the tissue with several of these round growths present.

Treatment with potassium iodide given internally in maximum doses, mercury in the form of protiodide, Fowler's solution with arsenic in gradually increasing doses up to tolerance, gave no beneficial prophylaxis. The various local antiseptics, as iodine, carbolic acid, bromine, bichloride of mercury, and several others, were applied externally, and some were injected into the lesion hypodermically but with no apparent relief. Both patients died.

In 1894 there were only two men in San Francisco who were equipped to do any bacteriological work, S. M. Mouser and Douglas W. Montgomery. The latter was called in on these cases, but could reach no definite conclusion as to the nature of these organisms. The consensus of opinion seemed to point to their being protozoa because of their resemblance to coccidia which cause the disease in rabbits and fowl known as "coccidiosis."

To determine the organism, some material was sent to W. H. Welch, of Johns Hopkins. Expressing doubt as to their protozoological nature, he turned the cultures over to T. C. Gilchrist, of the dermatology department, who had been working on blastomycosis, the lesion of which simulated those of the two cases noticed by Rixford and Thorne. Being unable, in turn, to determine the peculiar growths, he called in Stiles of Washington, an eminent medical zoologist and authority on protozoa. Perplexed as to this puzzling situation, Stiles named them "coccidioides" because of their similarity to the above-mentioned coccidia. Thus the organism from the first case he called *Coccidioides immitis* and that from the second, *Coccidioides pyogenes*.

Following this work, in which an appeal was made for reports

of similar cases, there was nothing additional until Posadas ('00) reported a case and Ophüls and Moffit, in conjunction with Ash (Phila. Med. Jour. 5: 1471, June 30, 1900), in the San Francisco Hospital, reported another. The three latter authors were able to show that on artificial substrates the organism grew out into long mycelia, like a typical fungus of the ordinary mold group. They could thus demonstrate that there were two dissimilar life-cycles present: one in the body of the individual having the spherical forms noted previously, and the other being the filamentous growth present on cultural media. It must be added, however, that Montgomery ('00) had formerly noticed the mold-like development on the cultures he had studied, but, believing them contaminations, had discarded the growths.

From this time on, various attempts were made to study the life history of the fungus, and of these the work of S. B. Wolbach ('04) is outstanding. For this particular point in the paper, however, it suffices to say that the organism has been sufficiently studied and investigated clinically, to arrive at a clearer understanding as to its recognition. There is still much work to be done as to its various other phases.

Etiology and Symptomatology of Coccidioidal granuloma

Coccidioidal granuloma, as a disease, belongs in the class of infectious granulomas. Its course may be exceedingly acute and end fatally in a few weeks, or the agony may be prolonged and a period of several years may elapse before death. Then again, the time may be long and drawn out, with slight pain, as is apparent in the case to be mentioned here. Between the two extremes of time, however, there is a sub-acute type which is neither rapidly fatal nor unduly prolonged in its progress, but which is characterized by a definite and positive tendency to widespread dissemination in the patient, with remissions and relapses, and in which the sufferer lives from a period of six months to two years after infection.

There are many clinical types involved in the disease, and in 1905 Ophüls classified three:

1. Primary cutaneous lesions and later generalization;
2. Primary pulmonary lesions and later generalization, but no skin lesions;

3. Primary pulmonary lesions and secondary subcutaneous lesions.

Many years later, Jacobson ('30) added three other clinical types, and with the addition of two more reported in the literature the total is eight:

4. Primary pelvic involvement without any skin lesions;
5. Primary meningeal or spinal cord involvement without any cutaneous or other skin lesions;
6. Primary involvement of the bones with secondary skin lesions;
7. Primary joint lesions;
8. Primary subcutaneous lesions.

In the past, much attention has been given to the cutaneous and subcutaneous types of the disease, due to the fact that the greater number of cases recognizable were of those kinds and hence of interest to the dermatologists. As a result, the literature comprises numerous papers on these various conditions with an intensive study of them. However, in the course of time, when other clinicians became interested because of the spread and fatality of the malady, it was noticed that pulmonary infection was very outstanding and more generalized. Ahlfeldt ('26) was able to show experimentally that both pulmonary and cutaneous infections may take place.

Numerous workers have demonstrated the entrance of the organism through an abrasion or some wound in the skin. Then, because of its frequent presence in a hot climate, such as is found in parts of California, particularly the San Joaquin Valley, the pulmonary type of infection is common, through the inhalation of the spores. It is very probable that such clinical types as mentioned previously, involving the meningeal, subcutaneous and joint lesions, in addition to the primary pulmonary and bone lesions, are primarily lung infections. This involvement may not be noticeable at first, but autopsy reports usually show positive results in these cases.

The disease is protean in its clinical manifestations, with the result that it resembles very closely various other infections, particularly tuberculosis. Its multiform clinical entities may simulate, through a metastatic action on the part of the fungus,

proliferating and suppurating processes, especially in the verrucous-like dermic lesions, as in the following: (a) The dermic lesions, which are nodular and ulcerative, in the form of painless, deep-seated, pinkish to dusky-red ulcers which become necrotic and sluggish, or may develop papillomatous growths, resembling epitheliomas, verrucas, tuberculosis in its various forms, syphilis, blastomycosis at times, and even sporotrichosis; (b) The subcutaneous coccidioidal involvement having three types of lesions —the flaccid tumor, the abscess, and the gummatous varieties may imitate cold abscesses or tumors as the names imply; (c) Pulmonary infections are usually diagnosed as pulmonary tuberculosis, but in many cases the correct diagnosis was determined post-mortem. Cases reported by Jacobson ('30), Montgomery ('00), Brown ('13), Hirsch ('23), and Taylor ('23) have given this information; (d) The osseous type of the disease has been confused with bone tuberculosis, osteomyelitis, or arthritis (Gardner, '04, Bowman, '19, Jacobson, '30). Hammack and Lacey ('24) found that twenty-one of twenty-three cases of generalized coccidioidal granuloma showed involvement of the bone. There is no roentgenological method of differentiating the disease from tuberculosis of the bone. Taylor ('23) suggested that "when bone destruction is particularly fulminating and when a proliferative process occurs along with the destruction, the diagnosis leans toward coccidioides rather than tuberculosis"; (e) Involvement of the meninges and the spinal cord usually requires a differential diagnosis from tuberculous meningitis, epidemic meningitis, and tumors of the spinal cord (Morris, '24, Rand, '30); (f) Gastro-intestinal disorders of coccidioidal granuloma require a thorough examination for the removal of the diagnosis of typhoid, as shown by Bowles ('12) and Carson and Cummins ('13). Cases reported along this line suggested very much the course usually taken by typical typhoid, and not until secondary cutaneous lesions had developed was an accurate diagnosis made; (g) Involvement of the lymph nodes usually suggests lymphatic leukemia, Hodgkin's disease, and lymphosarcoma. Ragle ('29) reported a case which early in its course resembled Hodgkin's disease and not until the cutaneous lesions appeared was the correct diagnosis given.

It would seem, therefore, that the diagnosis of coccidioidal granuloma is a difficult problem. Because of its close clinical association with tuberculosis, in order to establish the correct diagnosis it is necessary in every case to find the organism, in pus from cutaneous lesions or sinuses, in sputa, or in tissue from autopsy or biopsy material. Histopathological work does not prove anything unless the organism is seen. Frequently the lesions of coccidioidal granuloma show chronic inflammation without caseation, and some lesions may be purulent while others show the abundant necrosis typical of tuberculosis. At times, all these conditions may be present in the same section of tissue, so that it is essential that the fungus be sought. Single, negative, microscopic or cultural observations do not constitute a definite case against coccidioidal infection, for often in the presence of secondary pyogenic bacterial invaders repeated smears or cultures may be necessary before the organism can be found. Furthermore, inoculation in guinea pigs may be essential, and it is advised on all occasions.

Where the infection is of the primary visceral type with no opportunity for the study of either the tissue or the pus, the cutaneous allergic test has been applied. This consists of an intradermal injection of specific antigen obtained from a bouillon culture grown at 37° C. The filtrate of the culture, through a Berkefeld filter, contains the toxin which Jacobson ('28) claims to be an exotoxin. The principle of this reaction is that when the filtrate is injected cutaneously, a characteristic inflammatory reaction of the skin around the site of inoculation takes place in persons infected with the coccidioidal organism, whereas there is no toxic reaction in those free from the disease. This allergic cutaneous reaction may be due to an acquired cellular hypersensitiveness of the patient to the extracellular products of the organism, and the local manifestation is analogous to the tuberculin and luetin reactions. However, this method is suggested with caution and should be used only by trained investigators.

Positive tests have been reported by Davis ('24), Hirsch and Benson ('27), Jacobson ('28), and Chipman and Templeton ('30), while Cooke ('15) obtained negative results.

In addition to the types of involvement mentioned, autopsy reports reveal an enormous amount of pathological changes. These infectious conditions show the disseminating ability of the fungus. Of the great number of cases reported, the following conditions have been frequently observed:

Subcutaneous abscesses;
Lesions involving tissues of the head and neck;
Lesions involving the skin;
Meningitis and small granulomatous lesions within the brain substance;
Osteomyelitis of cranial bones with epidural or subcutaneous abscesses;
Oesophageal ulcer;
Lesions involving shoulder girdle or upper extremities;
Lesions of the thorax or its viscera;
Lesions involving the bony thorax (either ribs or sternum);
Miliary involvement of the lung;
Pneumonic consolidation simulating tuberculous pneumonia;
Fibrocaseous nodules without miliary involvement;
Caseation of hilar nodes;
Lesions involving the heart (pericarditis and endocarditis);
Lesions of the abdominal wall, spleen, liver, kidneys, pancreas, adrenals, iliac bones;
Lesions involving the whole genital apparatus, epididymus, and perirectal tissues;
Lesions of the bones of the pelvis or lower extremity;
Lesions involving the sacrum, both patellae;
Lesions in the region of the knee, and involving the ankles;
Erosion of the bodies of the vertebrae;
Involvement of the femoral, inguinal, retroperitoneal, mesenteric, lumbar, cervical, mediastinal, and peribronchial lymph nodes.

Agglutinins.—Immunological reactions have so far yielded unsatisfactory results. No agglutinins could be demonstrated by Cooke ('15), by Cummins and Sanders ('16) in experimentally infected animals, or by Davis ('24).

Precipitins.—Specific precipitins were demonstrated by Cooke ('15) in positive serum, in dilutions of 1 : 160, with an extract of

dried cultures of the organism as a precipitinogen, but negative results were obtained with the same antigen and normal serum or when specific immune serum was tested with an antigen similarly prepared from a blastomycetic organism. Positive results were obtained by other workers (Chipman and Templeton, '30), but in lower dilutions, while Cummins and Sanders ('16) report negative precipitin reactions.

Complement-Fixation.—Complement-fixation has been demonstrated with high concentrations of antigen by Davis ('24) and Chipman and Templeton ('30), whereas Cooke ('15) and Cummins and Sanders ('16) obtained negative results, although it must be pointed out that Cooke used a saline emulsion.

Specific Soluble Substance.—Hirsch and Benson ('27) and Hirsch and D'Andrea ('27a, '27b, '30) were able to demonstrate the specific soluble substance mentioned previously under the allergic reaction. Prolonged electro-dialysis of the filtrate from broth cultures causes the separation of white floccules. The dried specific substance is a white powder, not destroyed by heating to 80° C. for thirty minutes, readily soluble in water, physiologically saline, dilute alkalies (N/10 NaOH) and dilute acids (N/10 HCl), contains about 3–4 per cent nitrogen, and on hydrolysis 20–40 per cent reducing sugar measured as dextrose.

Mortality.—Coccidioidal granuloma has caused a great deal of excitement because of its high rate of mortality. Starting with as high as 100 per cent fatality, the number has gradually fallen until present reports show it to be approximately 65 per cent. As yet, this is a rather high percentage for all cases of infection, for it is probable that many cases do not reach the literature, or there may be many unrecognized mild cases, as pointed out before. Some cases may even have spontaneous recovery or become dormant and hence not noticeable. Faulty diagnosis may also be given as a reason for too few reports.

Treatment.—No wholly successful treatment of coccidioidal granuloma has been found. Recovery may be spontaneous, or death may come on slowly. Roentgen rays, surgery, iodides, and intravenous injection of crystal violet, arsphenamenes and tartar emetic have generally been unsuccessful. Antimony and potassium tartrate (Guy and Jacobs, '27) have been reported as successful by Tomlinson and Bancroft ('28) on a medical student

who had evidently contracted the disease while working on the organism. Jacobson ('27) used colloidal copper in conjunction with a special vaccine with encouraging results. Although his patients may be still alive, permanent cure is indefinite since remissions and relapses are apt to occur. In some cases, amputation of a limb has resulted in clinical cure.

Practically all races are affected, and of all the patients the greater number are males between the ages of 25 and 55. The higher percentage is in the agricultural class, the workers of the soil and its products.

Direct transmission from man to man or animal to animal has not been reported, although it is known that twenty-eight animals have had the disease. Besides, the organism has not until recently been isolated from the soil or vegetation where cases have occurred. *Coccidioides immitis* was obtained in cultures from the soil on the Delano Ranch in California where four cases had occurred among the Filipino working crew (Stewart and Meyer, '32).

Geographical Distribution.—The condition known as coccidioidal granuloma has a rather peculiar geographical distribution. Of the 286 cases reported prior to June 1, 1931, according to the bulletin issued by the California Department of Public Health ('31), 128 have been published, and a study of these cases shows that there is a decided concentration in California, in the San Joaquin Valley. Of this total number, 89.5 per cent or 254 cases originated in that state, the remaining cases having a widespread appearance as follows:[1]

South America	14	
Naples, Italy	2	
United States	16	
Arizona	2	
Colorado	3	
Illinois	2	
Kansas	1	
Missouri	1	(total now 2)
Nebraska	2	
Pennsylvania	1	
South Carolina	1	
Tennessee	1	
Texas	1	
Washington	1	

[1] Coccidioidal granuloma. State of Calif. Dept. Public Health, Spec. Bull. 57: 19. June, 1931.

As a result of its frequent presence in California, the term "California disease" has become the synonym of coccidioidal granuloma. It is hard to conceive how an organism which thrives so luxuriantly in animal tissues and which apparently finds the human body an excellent host and environment for its nutrition and propagation would willingly confine its activities to such large centers as Illinois, Pennsylvania, Missouri or Texas, to one or two persons, without manifesting any further evidence of its existence for a number of years. As mentioned previously, cases are occurring with rapid recovery or incorrect diagnosis, or the benign condition of many of the cases has not aroused enough interest in the attending physician to report the case. However, the disease has been made reportable, and it is hoped that all cases will be studied for future work.

Case Summary

The case involved in this report is worthy of note, since it is the second case, at the time of publication, known to occur in Missouri. However, due to certain factors, it is probable that the primary focus was California and not the above-named state.

In 1916, during his service in the Saint Louis City Hospital, Lipsitz, in connection with Lawson and Fessenden, reported a case of coccidioidal granuloma in the 'Journal of the American Medical Association.' Tuberculous broncho-pneumonia was at first given as the clinical diagnosis, but during the course of the disease numerous abscesses developed in the muscles, thus making it of a malignant nature. Death followed seven weeks after the onset of the symptoms. This patient had never been to California. This latter fact, coupled with the malignancy, made it an outstanding case.

For the second case, a brief history of which follows, the author wishes to express his gratitude to Dr. George Ives, of the Beaumont Clinic in Saint Louis, for the material and use of the data. The patient, a Saint Louis business man of about sixty years of age, visited California in August, 1927, and spent a short time in San Francisco and Los Angeles. Two years later, July, 1929, he developed a left-sided pleurisy with effusion, for which he was treated, and his recovery seemed complete. In April, 1931,

approximately two years later, a mild arthritis of the right knee developed. During the following summer (1931), he took sun baths at Atlantic City, but to no avail.

In September, 1931, the patient consulted Dr. Klinefelter, who found the Wassermann test negative, and the Schilling and leucocyte counts to be normal. However, the joint contained a considerable amount of fluid, the removal of which gave the patient considerable relief, although temporary. There is no edema, redness of adjacent skin, or apparent increased temperature of the skin. The patient is healthy and has gained weight since diagnosis, and the evidence, except as shown above, gives no clue to a diseased person.

In contrast to the first case which was extremely malignant, this one has shown a very mild course, and it is noteworthy that these two extremes should occur in the same locality.

The joint fluid showed a light-yellow, turbid color and small masses of fibrin, with some blood and 8000 leucocytes, approximately 75 per cent of which were polymorphonuclears. Smears for bacteria were negative.

A guinea pig was inoculated with the sediment of the fluid and watched for a month, at the end of which time an indurated lesion developed at the site of inoculation and both inguinal lymph nodes became enlarged. The disease was first diagnosed as tuberculosis, but when the autopsy revealed no tubercle bacilli but did show acid-fast, imperfectly spherical bodies with granular centers the possibility of blastomycosis set in. It was from this pig that the author obtained the culture for study.

Technique

To determine the morphology and to obtain cell measurements, mycelium was mounted in hanging-drop cultures and observed. In this manner, the various steps in mycelial development were also watched.

For cellular detail, transfers were made to a solution of glycerine (Merck C. P.) and crystal violet (1 per cent aqueous). The material was cleared and stained at the same time by this method, allowing for as rapid a diagnosis as is needed where many cultures are to be examined. The time necessary for the process varies

from 20 to 40 minutes; usually 30 minutes is sufficient, although in some cases only 10 or 15 minutes are needed.

Flemming's weak-killing and fixing solution was also used, being added directly to a tube of the culture. However, since the technique involved is long and the results are not materially different, it being necessary to stain and clear the material, steps which are extensive and time-taking, the former process was used almost exclusively.

Description

The fungus known as *Coccidioides immitis* has been shown by Ophüls and Moffit in 1900 to have two life-cycles: one in the host or tissue where it appears as a double-contoured cell varying from 5 to 60 μ in diameter and showing various amounts of granulation; the other on artificial culture media where it assumes a mold-like growth, with an intertwining network of mycelium composed of variously shaped, septate hyphae.

Inasmuch as the culture used in this paper was a mycelial growth on artificial media, the author had to content himself with an examination of the slides of tissue showing the round spheres, and wait for the development of the latter in anaerobic cultures. The forms observed, however, were typical of the classical cells.

In tissue, as stated above, the fungus grows and reproduces by endosporulation, a process which has caused a great deal of trouble in the classification of the organism. Numerous workers have made a study of this particular phase of the history of *Coccidioides immitis*, and Wernicke ('92) and Rixford and Gilchrist ('96), three of the first workers, gave the earliest description of the organism in tissue, the latter making the following statement:

> "The parasite, when fully grown, is enveloped in a distinct, double-contoured capsule, and then appears as an almost perfectly spherical organism. . . . These forms vary from 15–17 microns in diameter and consist of a thick, well-developed, spherical capsule, which can be deeply stained. Between the capsule and the contents is a clear, refractive layer which usually does not stain or is stained with difficulty. This clear zone appears homogeneous and structureless; it varies in thickness from 2 to 3 microns, but is hardly discernible when the organism is undergoing sporulation. The protoplasm surrounded by the clear layer stains very readily; it is for the most part finely granular, but contains also not a few scattered, coarse granules, sometimes arranged around the periphery, at other times entering into the formation of a network. . . . The 'protozoa' present in this case are reproduced by sporulation. . . . The number of

sporozoites which are finally developed from one organism varies, but is usually very large. During the process of sporulation, the capsule can be observed to become thinner and thinner until it consists only of a faint, but well-defined membrane which finally bursts. Just before this bursting stage, the organism changes its shape and assumes an oval form. The rupture takes place at one side or at both sides of the ovoid. One photomicrograph shows a number of fine prickles extending out from the capsule, especially at the sides."

It will be noticed that Gilchrist refers to the cells as 'protozoa.' This was the opinion held by several investigators until the mycological relationship was established in 1900.

Apparently, this description has been accepted, at least for the greater part, for we find that Ahlfeldt ('29) remarks: "We have found these prickles in several sections, and they are found only in adult organisms when they are ready to liberate young forms. We are able to confirm this method of sporulation, but think that the sporulating stage assumes an elliptical rather than an oval form."

Wolbach ('04) made a careful study of the life-cycle of *Coccidioides immitis* in tissue, pus, and sputum, and found that the contents of the cells may be "finely granular, almost homogeneous, or coarsely granular, reticulated, or vacuolated." The capsule may show radial striations, although usually homogeneous, a structure similar to that observed by Gilchrist and Ahlfeldt. He found that only the homogeneous cells segmented. The process begins with a division of the protoplasm peripherally, which extends inwards, forming many segments which are separated from one another by clear spaces. This is analogous to the mode of segmentation in a fertilized frog's egg, only here the divisions are simultaneous and not arranged in a series. The hyaline membrane then develops around the future spores. By the thinning out process of the capsule, the spores are liberated and held in groups by the growth inwards of the inflammatory tissue. That is apparently why so many of these encapsulated asci may be found in masses and not spread out evenly.

Inasmuch as the spores are non-motile, it is very likely that their spread in the tissue does not take place until the process of necrosis sets in, bringing about a moist condition. In this state of the lesion, the fungus finds its way into the lymphatic or blood streams and sets up endosporulation wherever held up, an act similar to metastasis.

Since the discovery that *Coccidioides immitis* had mold-like entities, several investigators have been able to demonstrate the change from the sphere to the mycelial growth and vice-versa. When placed on artificial media, the spheres send out thin, branched, septate hyphae. This phenomenon has been observed by Wolbach ('04) and MacNeal and Taylor ('14). The number of filaments so developing is indefinite, and, if we accept Wolbach's report, come apparently from the capsule, since he states that "the protoplasm meanwhile may remain shrunken within the capsule, and without demonstrable connection with the growing filaments." This occurrence, however, is opposed to that found by the latter authors who make the following statement:

> "The capsule was penetrated at several points by blunt, protoplasmic outgrowths from the interior protoplasm. The rapidity of development of these shoots in some instances was such as to give the impression of ameboid movement at the growing tips. The shoots, at first naked cylinders of granular protoplasm, soon produced a definite, more hyaline wall about them, branched abundantly and irregularly and developed septa. After several hours, ovoid bodies of more homogeneous structure were numerous within many of the cells. After 24 hours, the circular colony had attained a diameter of one millimeter, and was made up of branched, interlacing, septate threads from 2 to 8 microns in diameter, with the old capsule of the original sphere as the center."

This latter quotation is in accord with observations made by the author on these endogenous, sporulating asci obtained in anaerobic cultures and grown on nutrient agar. The phenomenon is rather apparent, since an ascus which had fully matured and ruptured allowed the spores to spread out and develop (pl. 25, figs. 1–4). It will be seen that the spore elongates, becomes branched, with cross-walls, and the mycelial characteristics of the common molds, the varied hyphae measuring from 1 to 1½ μ in width, become abundant.

As the hyphae develop, they become wider, depending on the media on which grown, but averaging 2 to 2½ μ, being as much as 4 μ in width on some substrates. The wall becomes thicker and swellings arise on the filaments, these latter tending to form the racquet mycelium characteristic of *Coccidioides immitis* and suggestive of *Endomyces capsulatus* and the *Trichophytons* (figs. 5–12). With this increase in size the cytological detail becomes complex, and a number of deep-staining, round masses are seen which represent the nuclei and future spores. Hyphal enlargements,

measuring approximately 5 × 11 μ, become abundant as the culture gets older. These enlargements have large amounts of densely stained material representing the beginning of chlamydospore development (figs. 7–8).

With greater age there is an increased growth in width of the hyphae, the cell walls become thicker, and then there is a resorption of cytoplasm (fig. 12), representing the beginning of arthrospore formation seen in figs. 13 and 14, and the development of spores. The cell membrane becomes clear, showing a hyaline space between the inner and outer walls. The cell contents change from an irregular, granular mass to a smooth, homogeneous substance, followed by the formation of spores with a clear space around each. In the meantime, however, the structure is changing from a rather rectangular cell to a sphere. The cytoplasm, representing the connecting link between each of the arthrospores, now becoming chlamydospores and then asci, has changed from a smooth cell to a very clear membrane which either becomes absorbed by the adjacent cell wall, or completely disintegrates. From observations, it is believed that the latter of the two is the more probable. In any case the cells tend to become round (figs. 18 and 19) and may form chains or be single. On artificial media, the tendency is for the cells to remain in chains when young, and to become large and single when the culture is older.

It usually requires about 8 to 10 days for these large spheres or asci to form in a definite amount on an agar substrate, this being in accordance with the work of Ahlfeldt ('29), and several others (Ophüls, '05, Wolbach, '04, MacNeal and Taylor, '14). At the end of several weeks, the colony has become fluffy and aerial hyphae are in abundance, the asci being numerous and loose. The medium assumes a brownish color imparted to it by the mycelium which has turned to a smoky brown.

Several workers have attempted to connect both phases in the life-cycle of *Coccidioides*. Wolbach ('04) and Ophüls ('05) found similar results in that a sphere developed from a segment of the mycelium, observations compatible with those made by the author in anaerobic cultures. The work of these men consisted of injecting mycelium into animals and watching the growth. In tissue and in body fluid, within the individual, chains are very

few and the single endosporulating bodies are large. These organisms then go through the cycle described.

CULTURAL DESCRIPTIONS

The culture furnished in this study was growing on a Sabouraud's agar slant. Colonies were many and small, spreading over the surface of the tube. Transfers were made to a series of media ranging from the more highly concentrated hydrogen ions to the less concentrated, on the pH scale roughly from 4.00 to 7.53. All cultures were grown at 25° C.

Because of its presence in a human lesion, it was thought best to use media which contained protein as a source for nitrogen. It was found, however, that the organism grew quite well on the simplest carbohydrate media as well as on agar nutrients. This is in accord with the work of Ophüls ('05) and of others, especially that of Bump ('25). In general, it was found that the organism grew quite well on a wide pH range, but the number of reproductive bodies was greatest on protein media. Inasmuch as the fungus can be identified by its microscopic morphology on various media, no particular attention was paid to its fermentative abilities at that time (Proescher, Ryan, and Krueger, '26).

Following are descriptions of colonies on several of the media on which determinations were made. These are arranged in the order of decreasing concentration of hydrogen ions.

Raulin's Solution (*pH 4.15*).—Colony white, filamentous, growing in large flakes partly submerged in the medium. Hyphae 1½ μ in width, long, few cross-walls, with swollen portions 2½ × 6 μ. Older hyphae show few chlamydospores and arthrospores.

Richard's Solution (*pH 4.36*).—Growth similar to that on Raulin's solution, mycelial growth being more abundant. Colony gray-white in color and in large filamentous flakes. Hyphae very thin, ½ to 1 μ in width, showing hyphal swellings and a few chlamydospores.

Czapek's Agar (*pH 4.43*).—Mycelium white, loose, cottony in growth. Hyphae 1½ to 2 μ wide, with chlamydospores and swellings suggestive of the racquet mycelium of *Trichophyton*.

Malt Extract Agar (*pH 5.20*).—Colony creamy-white in color, becoming brown after several weeks. Growth loose and cottony,

thick at the inoculum, forming concentric circles, and attaining a diameter of 9 cm. in 5 weeks. Hyphal swellings present, round, thick-walled chlamydospores in abundance, suggestive of the "*endoconidies*" of Vuillemin ('99), and the "*globules internes*" of Salvat and Fontoynont ('22). These measure from 4 to 7 μ in the culture.

Sabouraud's Agar (*pH 5.60*).—Growth rapid, cream-colored when young, becoming light brown with age. Mycelium thick, cottony, reaching a diameter of 8½ cm. in 5 weeks. Hyphae long, growing up on the side of the tube, attaining a width of 2½ μ in mature stage. Abundance of chlamydospores and arthrospores in older colonies, having various sizes and shapes. Hyphal swellings also present and numerous.

Oat-Meal Agar (*pH 5.80*).—Growth very loose and cottony, color white, not turning brown with age. Hyphal measurements similar to that on Sabouraud's agar.

Corn-Meal Agar (*pH 6.00*).—Growth loose and cottony, similar to that on oat-meal agar, reaching a diameter of 8 cm. in 5 weeks. Mycelium loose around inoculum, followed by a heavier growth. Hyphae 1½ μ in width, forming swellings 2½ to 3½ μ in width and 4½ μ in length to the normal hypha. Arthrosporous formation abundant in the older colonies.

Gelatin (*pH 6.70*).—Medium liquefied. Growth cone-shaped, with loose, cottony, branching, septate mycelium 2 μ in width, penetrating to a depth of 2.4 cm. in 5 weeks. Mycelium as on other media.

Beef Extract Agar (*pH 6.81*).—Growth loose and cottony, spread over surface of plate, reaching a diameter of 9 cm. at the end of 5 weeks. Color white, becoming brown with age. Abundance of thick-walled cells. Measurements same as on Sabouraud's agar.

Glycerine Agar (*pH 7.00*).—This medium consists of beef-extract agar with 6 per cent glycerine. Growth most favorable on this agar, with an abundance of spores (arthro- and chlamydo-), at first thick at inoculum with a thinner area surrounding it, that being encircled by a thick elevated mass; size of colony reaching a diameter of 3 cm. in 7 days and 8 cm. in 5 weeks. Hyphae thicker than on other media, having a width of 2½ to 3 μ, with the swollen parts attaining a width of 7 μ.

Eosine-Methylene-Blue Agar (*Product of the Digestive Ferments Co., pH 7.00*).—This medium was used merely as a part of a routine. Growth characteristic of the group, similar to that on Sabouraud's agar, with a diameter of 8 cm. in 5 weeks. Hyphae 2 μ wide, septate and branching, with the characteristic swellings and the formation of chlamydospores in the older colonies. Colony assumes a pink color which spreads over the mycelium, completely halting growth after 6 weeks.

Nutrient Agar (*Product of the Digestive Ferments Co., pH 7.27*).—Growth similar to that on beef-extract agar. Diameter of colony 9 cm. at end of 5 weeks.

Endo's Medium (*pH 7.53*).—Growth very slow, dye of medium being absorbed by inoculum, with growth stopping after 2 weeks.

Anaerobic Media.—Liborius' method of anaerobic cultivation was used.[2] Growth present, but poor, as contrasted with that in aerobic circumstances. The organism thus shows a facultative aerobic condition.

Summary of Cultural Work

The organism involved in the case mentioned previously was found to be characteristic of the fungus, *Coccidioides immitis*, based on the following properties, both culturally and morphologically, as seen in the experimental work:

1. Ability to grow on a wide variety of media as indicated.
2. Occurrence on a wide range of pH.
3. Color changes to a light brown with age.
4. Characteristic flaky growth on liquid media.
5. Condition of facultative aerobiosis.
6. Branching, septate mycelium.
7. Peculiar hyphal swellings.
8. Characteristic mycelial measurements.
9. Formation of the chlamydospores.
10. Formation of ascogenous cells.
12. Endogenous spore formation in anaerobic conditions.

[2] Method taken from Hiss-Zinsser, Textbook of Bacteriology, p. 146. New York, 1929.

Discussion

As many as are the terms applied to the disease, so varied are the names that have been used in designating the organism. If we go back to the time when the fungus was first found (Wernicke, '92), we would notice that it was called a protozoon. In fact, so great was the belief in its zoological affinity that when, some time later, two more cases were observed (Rixford and Gilchrist, '96) the medical zoologist, Dr. Stiles, was called in to render a diagnosis. He called the organism of the second case *Coccidioides immitis*, because of its resemblance to a protozoon, and that of the third case *Coccidioides pyogenes*.

Several years later, in 1900, Ophüls and Moffit, in conjunction with Ash, found that the organism was similar to a mold, and gave it the name *Oidium coccidioides*, and referred it to the class Ascomycetes. It was also termed *Oidium protozoides*.

Ricketts ('01) studied seventeen organisms and concluded that they belonged to the genus *Oidium*, distinguishing the following types as varieties: (1) Blastomycetoid or yeast-like; (2) Oidium-like; (3) Hyphomycetoid.

Verdun in 1907 prefers to call the fungus *Oidium immite*, while Brumpt ('27) insists on its classification as a hyphomycete, *Mycoderma immitis*, and to make matters more complicated, Castellani ('28) renames the genus *Blastomycoides*.

The classification of *Coccidioides immitis* has been very uncertain and the names applied to the organism very indefinite, with the result that no established taxonomic position has been assigned to it. There are those who assume that it is a hyphomycete, with definite hyphomycetous characters, but the position of these authors is very shaky as we shall see. On the other hand, many believe it to be an ascomycete, showing ascus formation and definite mycelial characteristics, and it is in this group that the classification here to be established is involved. To avoid any confusion, the California Department of Public Health has named it "fungus coccidioides," which makes an additional term to deal with.

An analysis of these terms will immediately eliminate several of them. In the first place, there is no evidence of budding in any of its forms. In the tissue, as described previously, spore forma-

tion occurs chiefly through endosporulation. In some cases, however, instances have been observed where the condition simulated budding very much, in that two spheres accidentally grew together with adjacent surfaces attached, but these cells had never become detached while young. Old cultures usually show a similar condition when two or several segments of the mycelium have never become separated by the disintegration of the cell structure between them (pl. 25, fig. 18). This phenomenon has led many to refer the organism to the genus *Oidium*, but since budding is absent from the life-cycle of *Coccidioides immitis*, it cannot be included in the group where this type of reproduction may occur.

Also, a glance at the development of mycelium and the formation of the arthrospores and then chlamydospores, producing what the author prefers to call asci, shows that they are not oidia. Thus the term blastomycete, as used in its literal sense, would be void here. Furthermore, the term *Mycoderma*, as defined by Brumpt, is equivalent to *Oidium*, and must be eliminated, since the fungus reproduces in the tissue by budding and never by endogenous spore formation. Brumpt now prefers to designate *Coccidioides immitis* as belonging to the Chytridiales of the Archimycetes. On examining the group, this view would seem a good deal more logical than the former conception, but the characteristic septate hyphae present in *Coccidioides* are lacking in the chytrids, hence that idea is amiss.

In Vuillemin's classification of the Fungi Imperfecti ('10), the groups are divided on the basis of their reproductive methods or on the kind and manner of spore formation. Thus in one of the orders, Thallosporales, the cells, thallospores, formed by the vegetative mycelium, are of a vegetative nature and not particularly suited for reproduction. Here we find accordingly two suborders: (1) the Blastosporineae which reproduce by blastospores, including the budding yeasts or yeast-like fungi, as *Monilia;* (2) the Arthrosporineae, the group reproducing by arthrospores. This latter group includes such forms as *Oidium lactis* and comprises the Actinomycetes. Two families are present: (1) the Mycodermaceae, with mycelium simulating that found in the first suborder, and; (2) the Nocardiaceae, the Actinomycetes.

Of the latter two categories mentioned above, the Mycoder-

maceae have some interest here, but, in the first place, the mycelium is monilia-like, and in the second, the arthrospores referred to in *Coccidioides* are not of that nature, *sensu stricto*, but represent an early stage in the formation of asci. Furthermore, the characteristics of *Oidium lactis* are not found in the above group. On closer study, one finds that the group classified by Vuillemin as Thallosporales has several features in common with those of the Ascomycetes, following Gaümann and Dodge ('28). As a family, however, the Mycodermaceae include too many budding forms, and that condition would eliminate the transfer of *Coccidioides* to that category.

Several writers relegate *Coccidioides* to a generic position in the Protomycetaceae (Fonseca et Arêa Leão, '28, Basgal, '31), to which they have also transferred *Endogone*, a member of the Endogonaceae of the Zygomycetes. It must be said here, however, that these authors have dealt with the organism occurring in South America. Almeida ('28) has summarized the principle features, on a comparative basis, of the organism found in the United States and the one present in Brazil, which, if we were to accept as significant of the fungus of the latter country, should be considered as different from *Coccidioides immitis* only as a species. This Brazilian fungus is similar to that described by Posadas, and it has been named *C. brasiliensis* by Splendore in 1912, emend. F. Almeida, but the term *Posadasia esferiformis* Canton 1898 has been used to designate the fungus of that region. In view of present knowledge, however, *Posadasia* is in synonymy with *Coccidioides*, and because of priority, *esferiformis* should be used as a specific name instead of *brasiliensis*. The term *C. esferiformis* would thus constitute a species of the same genus, in the same family. In referring the genus to the Protomycetaceae, the characters of two diverse groups have been intermingled, without indicating any connecting links. Furthermore, an examination of the Protomycetaceae (Büren, '15) shows that sexuality is prominent. Although showing similarity in some degree in the mycelial characteristics, as the chlamydospores, intercalary cells and branching, phenomena which may be present in a great many other fungi, still, when we approach the essential point of differentiation, the reproductive process, the act of spore development

and dissemination pursues a different course. In the Protomycetaceae, with increasing age, the spores develop as a sheath on the inner surface of the third layer which elongates, breaks through the outer two coverings, accumulates the spores in a mass, and then shoots the aggregation into space.

Following this description of the Protomycetaceae, Mazza and Parodi ('28) have established a new genus, *Pseudococcidioides*, with properties similar to those found in *Coccidioides*. Since the growth on agar is not very well defined no exact classification can be established for this organism, but inasmuch as the whole process suggests that found in other members of the Protomycetaceae, particularly in the mode of formation of the spores and the presence of vacuoles, a tentative position as a new genus in that family may be assumed.

A reference must also be made to the genus *Rhinosporidium* which was established as a fungus by Ashworth in 1923, and which has been associated with the above genera in the family Protomycetaceae. This organism forms an ascocarp with a diameter up to 0.8 mm. Within this structure there is a great number of asci which are called pansporoblasts or morulas, which are liberated by the rupture of the ascocarp, each measuring 6 μ and containing 4 to 16 spores. An encapsulation of the organism by mononuclear leucocytes follows and then the process of growth continues. Several attempts to grow the cells on media have given no results, hence the determination as a fungus and the classification among the Phycomycetes, after Ashworth ('23), more specifically among the Olpidiaceae of the Chytridiales as Brumpt prefers it, or in the Protomycetaceae (Fonseca, '28), must of necessity rest indefinite in view of the scanty criteria present.

With this brief discussion of the conceptions of the taxonomic position of *Coccidioides*, let us turn to a consideration of the phylogenetic possibilities.

In the first place, it would seem that we are dealing with a very old organism. Here we have a fungus which has become so adapted to its parasitized host that it is able to go through a whole life cycle without a change of habitat. Of course, several steps have been eliminated in the process, but when we realize

that the end result is the same, *Coccidioides* must be considered of extreme senescence. A second proof rests on the fact that the sexual process has degenerated to the state where copulation is lost and parthenogamy prevails. A condition of this sort may be found in certain of the Hemiascomycetes where the mode of development simulates very much that of the Protomycetaceae, except that the former have copulation, either iso- or heterogamous with a definite number of spores, an advanced character, while the latter have the copulation of spores. The group constituting the former category, the Endomycetaceae, is significant in that it has been treated by many as having definite copulating forms. However, an examination of its phylogenetic relationships establishes two developmental series, that is, the isogamous, as found in *Eremascus fertilis*, and the heterogamous, as found in *Endomyces capsulatus*, or *E. Magnusii*. Both series may end in parthenogamy. This latter fact is of extreme significance here, because an association of these characters helps to establish the position of *Coccidioides*.

After due consideration of the characters presented by the members of the Hemiascomycetes and a study of the criteria involved, it appears to the author that the organism in question, namely, *Coccidioides immitis*, should be placed in the Endomycetales. Having a relationship to the Zygomycetes, on the one hand, and a semblance to the Taphrinales with the Protomycetaceae, on the other, *Coccidioides* constitutes a division comprising the affinities of both. Receiving several of its features from the Endomycetaceae and others from the Saccharomycetaceae and Protomycetaceae, makes it necessary, in view of such facts, to establish a new family, Coccidioideaceae, with *Coccidioides* as the principle genus, and to place that family in a position following the Endomycetaceae and preceding the Saccharomycetaceae. Such a division would represent the parthenogamous end of the copulation series. Although difficult to conclude definitely as to which of the two mentioned previously this fungus may belong, nevertheless, judging by the particular mode of formation of the asci, isogamy, in all probability, had prevailed.

The life cycle of *Coccidioides immitis* may be shown diagrammatically, as follows:

(a) in the tissue,

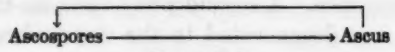

Diagram I.

(b) on artificial media,

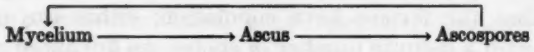

Diagram II

In the following diagram, the position of the new family in relation to the now-existing divisions may be easily noticed:

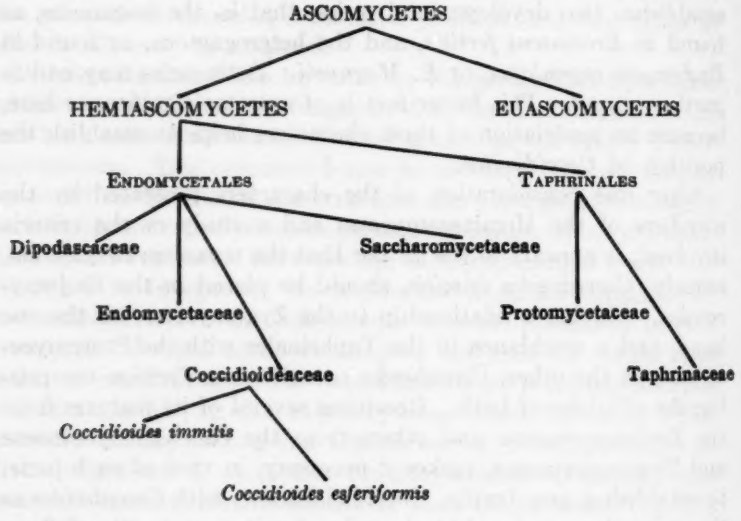

Diagram III

Coccidioideaceae Moore, new family.

This family differs from the others of the Endomycetales and Taphrinales in that the sexual development is completely lost and parthenogamy prevails; budding in any of its forms is lacking; an entire life-cycle is present in the parasitized host; endogenous,

sporulating asci arise as a result of the disintegration of the intervening cells; and finally, the number and form of development of the spores is different.

Coccidioideaceae Moore, fam. nov.

Mycelium in culturis abundans sed in hospite fere deest; asci parthenogetici singuli ex cellulis hypharum degeneratione cellularum intermediarum orti, sporae in asco multae parvae ellipsoideae, sphericaeve.

Ab Endomycetaceis sexus gemmationisque carentia, sporarum in asco multitudine, a Saccharomycetaceis mycelii abundantia differt.

Coccidioides Stiles 1896.

Growth in tissue by endogenous spore formation with many spores, round to oval; on artificial media there is an abundance of mycelium varying with the type of agar used. Hyphae septate and branching, measuring from ½ to 4 μ in diameter, characteristic hyphal swellings and formation of terminal hypnospores. Budding entirely lacking, as well as copulation. Asci form from the hyphae through the differentiation of cellular material and the distintegration of intervening cell membranes, varying from 1 to 80 μ in diameter.

Coccidioides immitis Stiles 1896.

Development by endogenous spore formation in tissue, in asci varying from 4 to 80 μ in diameter; spores numerous, round to oval. Budding and copulation lacking. On agar, colony of abundant mycelium varying from a light pink when young to a smoky-brown with age. Hyphae septate and branching, ½ to 4 μ in diameter on different media. Hyphal swellings varying from 2½ to 7 μ in width and 5 to 12 μ in length. Chlamydospores abundant, 5 to 8 μ in diameter. Terminal hypnospores several, approximately 5 × 8 μ.

Coccidioides esferiformis (Canton 1898) Moore, n. comb.

Reproduction in tissue by dehiscence of spores from the interior through the membrane, until death of cell. Asci vary from 1 to 40 μ in diameter. Growth on media difficult, optimum at pH 7.4 after 20 days. Colony white to gray. Cultural characteristics otherwise similar to the above.

With the establishment of *Coccidioides esferiformis* as a species differing from *C. immitis*, the percentage of cases occurring in California prior to June 1, 1931, plus the present case, would rise to 93 per cent. It is probable that the cases found outside of California may have had some traffic with that State as is evident from the patient here examined, and if so it shows the fungus to be endemic to the above-named region.

SUMMARY AND CONCLUSIONS

1. The history of coccidioidal granuloma is given, with a review of the early diagnosis of the organism.

2. The etiology and symptomatology show various involvements and complications and eight clinical types.

3. The immunological reactions show an incompleteness in definite beneficial results.

4. The cases show a peculiar geographical distribution, with a localization of 93 per cent of the cases due to *Coccidioides immitis* in California, and the remainder spread throughout the country and 2 cases in Italy, while many cases due to *C. esferiformis* have occurred in South America.

5. A summary of the second case known to occur in Missouri is given, showing its extremely benign course as compared with that of the first.

6. The fungus is described in detail, showing its double life-cycle: one in the tissue as a sphere, and the other on artificial media as a mold-like growth. A study of the organism reveals its relationship to the group characteristic of the Endomycetales.

7. The organism was grown on various media, showing a wide range of properties typical of *C. immitis*.

8. The phylogeny and classification of the organism is discussed, with the result that a new family, Coccidioideaceae, is established and placed in a position between the Endomycetaceae and the Saccharomycetaceae, having the affinities of both. One genus and two species are at present recognized.

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Bibliography

Ahlfeldt, F. E. ('26). Studies in coccidioidal granuloma: II Mode of infection. Arch. Path. & Lab. Med. **2**: 206–216. 1926.

———, ('29). Special observations on the morphology of Coccidioides immitis. Jour. Inf. Dis. **44**: 277–281. 1929.

Almeida, F. P. de. ('28). Estudo comparativo do granuloma coccidioidico nos Estados Unidos e no Brasil. Facul. Med. de São Paulo, Ann. **3**: 91–99. 1928.

Ashworth, J. H. ('23). On Rhinosporidium seeberi (Wernicke 1903) with special reference to its sporulation and affinities. Roy. Soc. Edinb., Trans. **53**: 301–342. 1923.

Basgal, W. ('31). Contribuição ao estudo das blastomycoses pulmonares. Doctorate thesis in medicine. Rio de Janeiro, 1931.

Beck, M. D. ('29). Occurrence of Coccidioides immitis in lesions of slaughtered animals. Soc. Exp. Biol. & Med., Proc. **26**: 534–536. 1929.

Bowles, F. H. ('12). Coccidioidal granuloma. Am. Med. Assoc., Jour. **59**: 2253. 1912.

Bowman, W. B. ('19). Coccidioidal granuloma. Am. Jour. Roentgenol. **6**: 547–555. 1919.

Brown, P. K. ('07). Coccidioidal granuloma: Review of the eighteen cases and reports of cases fifteen and sixteen. Am. Med. Assoc., Jour. **48**: 743–746. 1907.

———, ('13). A fatal case of coccidioidal granuloma. *Ibid.* **61**: 770–771. 1913.

———, and Cummins, W. T. ('15). I. A differential study of coccidioidal granuloma and blastomycosis. II. Report of two additional cases of coccidioidal disease. Arch. Int. Med. **15**: 608–627. 1915.

Brumpt, E. ('27). Précis de parasitologie. pp. 1213, 1383. Masson et Cie. Paris, 1927.

Bump, W. S. ('25). Observations on growth of Coccidioides immitis. Jour. Inf. Dis. **36**: 561–565. 1925.

Büren, G. von. ('15). Die schweizerischen Protomycetaceen mit Besonderer Berücksichtigung ihrer Entwicklungsgeschichte und Biologie. Doct. Thes. Univ. Bern. 1915.

Burkhead, C. E. ('22). Oidiomycosis, including one case of coccidioidal granuloma and one of cutaneous Blastomycosis. Kan. Med. Soc., Jour. **22**: 101. 1922.

California Department of Public Health. ('31). Coccidioidal granuloma. Cal. Dept. Health, Special Bull. 57. 1931.

Carson, G. R., and W. T. Cummins ('13). A case of coccidioidal granuloma (California Disease). Am. Med. Assoc., Jour. **61**: 191–192. 1913.

Castellani, A. ('28). Fungi and fungous diseases. Am. Med. Assoc. Chicago, 1928.
Chipman, E. D. ('13). The newer cutaneous mycoses. Am. Med. Assoc., Jour. 61: 407–412. 1913.
———, and Templeton, H. J. ('30). Coccidioidal granuloma. Arch. Derm. & Syphil. 21: 259–275. 1930.
Cooke, J. V. ('15). Immunity tests in coccidioidal granuloma. Arch. Int. Med. 15: 479–486. 1915.
Cummins, W. T. ('28). Coccidioidal granuloma. Calif. & West. Med. 29: 265. 1928.
———, and J. Sanders ('16). The pathology, bacteriology, and serology of coccidioidal granuloma, with a report of two additional cases. Jour. Med. Res. 35 (N. S. 30): 243–257. 1916.
———, Smith, J. K., and C. H. Halliday. ('29). Coccidioidal granuloma: An epidemiologic survey, with a report of 24 additional cases. Am. Med. Assoc., Jour. 93: 1046–1048. 1929.
Davis, D. J. ('24). Coccidioidal granuloma: With certain serologic and experimental observations. Arch. Derm. & Syphil. 9: 577–587. 1924.
Dickson, E. C. ('15). Oidiomycosis in California, with especial reference to coccidioidal granuloma. Arch. Int. Med. 16: 1028–1044. 1915.
———, ('29). Mimicry of tuberculosis by coccidioidal granuloma. Assoc. Am. Physicians, Trans. 44: 284–294. 1929.
Evans, N. ('09). Coccidioidal granuloma and blastomycosis in the central nervous system. Jour. Inf. Dis. 6: 523–536. 1909.
———, and Ball, H. A. ('29). Coccidioidal granuloma: Analysis of 50 cases. Am. Med. Assoc., Jour. 93: 1881–1885. 1929.
Fonseca, O. da ('28). Ensaya de revisión de las blastomicosis sud americanos. Inst. Clin. Quirurg., Bol. 4: 469–502. 1928.
———, ('30). Besonders Studium des Coccidioides immitis und des coccidioidischen Granulomas. Seuchenbekämpfung, Ätiologie, Prophylaxe und Therapie der Infektionskrankheiten der Menschen und Tiere. 7[4]: 237–250. 1930.
———, et Arêa Leão, A. E. de ('28). Sur le granulome coccidioidal. Formes d'evolution du parasite dans les tissus, dans le pus des ganglion lymphatiques, et dans les cultures. Position systematique du "Coccidioides immitis." Compt. Rend. Soc. Biol. 98: 619–621. 1928.
Gardner, S. J. ('04). An unusual infection in the bones of the foot. Calif. State Jour. Med. 2: 386. 1904.
Gaûmann, E. A., and Dodge, C. W. ('28). Comparative morphology of fungi. pp. 127–165. McGraw, Hill Book Co. First edition. New York, 1928.
Giltner, L. T. ('18). Occurrence of coccidioidal granuloma (oidiomycosis) in cattle. Jour. Agr. Res. 14: 533–541. 1918.
Guilliermond, A. ('07). A propos de l'origine des levûres. Ann. Myc. 5: 49–69. 1907.
———, ('09). Recherches cytologiques et taxonomiques sur les Endomycétacées. Rev. gén. Bot. 21: 353–391, 401–419. 1909.
———, et G. Péju ('19). Sur un nouveau champignon présentant des caractères intermédiaires entre les levures et les Endomyces. Compt. Rend. Soc. Biol. 82: 1343–1345. 1919.
Guy, W. H., and F. M. Jacobs ('27). Granuloma coccidioides. Arch. Derm. & Syphil. 16: 308–311. 1927.

Hammack, R. W., and J. M. Lacey ('24). Coccidioidal granuloma in southern California. Calif. & West. Med. **22**: 224–226. 1924.

Hektoen, L. ('07). Systemic blastomycosis and coccidioidal granuloma. Am. Med. Assoc., Jour. **49**: 1071–1077. 1907.

Helsley, G. F. ('19). Coccidioidal granuloma: Report of a case. *Ibid.* **73**: 1697. 1919.

Henrici, A. T. ('30). Molds, yeasts and actinomycetes. John Wiley and Sons, Inc. New York, 1930.

Hirsch, E. F. ('23). Introduction of coccidioidal granuloma into Chicago. Am. Med. Assoc., Jour. **81**: 375–377. 1923.

———, and H. Benson ('27). Specific skin and testis reactions with culture filtrates of Coccidioides immitis. Jour. Inf. Dis. **40**: 629–633. 1927.

———, and D. D'Andrea ('27a). The specific substance of Coccidioides immitis. *Ibid.* 634–637. 1927.

———, ———, ('27b). Sensitization of guinea-pigs with broth culture filtrates and with killed mycelium of Coccidioides immitis. *Ibid.* 638–640. 1927.

———, ———, ('30). Allergic testis reactions in guinea-pigs with coccidioidal granuloma. Jour. Immun. **18**: 121–125. 1930.

Ives, G. ('32). A case of coccidioidal granuloma. St. Louis Med. Soc., Weekly Bull. **26**: 290. 1932.

Jacobson, H. P. ('27). Granuloma coccidioides—apparently successfully treated with colloidal copper. Calif. & West. Med. **27**: 360–364. 1927.

———, ('28). Coccidioidal granuloma: Specific allergic cutaneous reaction: Experimental and clinical investigations. Arch. Derm. & Syphil. **18**: 562–567. 1928.

———, ('28a). Coccidioidal granuloma. Calif. & West. Med. **29**: 392–396. 1928.

———, ('29). Coccidioidal granuloma, further observations with report of seven additional cases. Med. Jour. & Rec. **130**: 424, 498. 1929.

———, ('30). Coccidioidal granuloma: A clinical and experimental review with case reports. Arch. Derm. & Syphil. **21**: 790–814. 1930.

Jaffé, R. H. ('30). Microscopic changes in coccidioidal granuloma. Virch. Arch. f. path. Anat. **278**: 42–61. 1930.

Lemon, W. S. ('29). Clinical manifestations of coccidioidal granuloma. Mayo Clinic, Proc. Staff Meet. **4**: 305–306. 1929.

Lindau, G. ('00). Sphaeriales. In Engler & Prantl's Natürlichen Pflanzenfamilien. I Teil. Abt. **1**: 415. 1900.

Lipsitz, S. T., G. W. Lawson, and E. M. Fessenden ('16). Case of coccidioidal granuloma. Am. Med. Assoc., Jour. **66**: 1365–1367. 1916.

Lynch, K. M. ('20). Coccidioidal granuloma, including the first reported case east of the Mississippi. South. Med. Jour. **13**: 246–249. 1920.

MacNeal, W. J., and C. E. Hjelm ('13). Note on a mold, Coccidioides immitis, found in a case of generalized infection in man. Am. Med. Assoc., Jour. **61**: 2044. 1913.

———, and R. M. Taylor ('14). Coccidioides immitis and coccidioidal granuloma. Jour. Med. Res. **30** (N. S. **25**): 261–274. 1914.

Mazza, S., y S. Parodi ('28). Una micoses chaqueña de la laringe causada por un nuevo tipo de hongo. Inst. Clin. Quirurg., Bol. **4**: 539–544. 1928.

Montgomery, D. W. ('00). A disease caused by a fungus: the protozoic dermatitis of Rixford and Gilchrist. Brit. Jour. Derm. **12**: 343–350. 1900.

———, and H. Morrow ('04). Reasons for considering dermatitis coccidioides an independent disease. Jour. Cut. Dis. **22**: 368. 1904.

———, and O. S. Ormsby ('08). Systemic blastomycosis: Its etiologic, pathologic, and clinical features as established by a critical survey and summary of twenty-two cases, seven previously unpublished: The relation of blastomycosis to coccidioidal granuloma. Arch. Int. Med. **2**: 1–41. 1908.

———, H. A. L. Ryfkogel, and H. Morrow ('03). Dermatitis coccidioides. *Ibid.* **21**: 5–10. 1903.

Morris, M. ('24). Coccidioides of the central nervous system. Calif. & West. Med. **22**: 483–484. 1924.

Morris, R. T. ('13). A case of systemic blastomycosis. Am. Med. Assoc., Jour. **61**: 2043–2044. 1913.

Ophüls, W. ('05). Coccidioidal granuloma. *Ibid.* **45**: 1291–1296. 1905.

———, ('05a). Further observations on a pathogenic mold formerly described as a protozoan (Coccidioides immitis, Coccidioides pyogenes). Jour. Exp. Med. **6**: 443–486. 1905.

Posadas, A. ('00). Psorospermiose: Infectant généralisée. Rev. de Chir. **21**: 276–282. 1900.

Proescher, F., F. Ryan, and A. P. Krueger ('26). Report of a case of coccidioidal granuloma with autopsy findings. Jour. Lab. & Clin. Med. **12**: 57–70. 1926.

Pulford, D. S., and E. E. Larson ('29). Coccidioidal granuloma: Report of a case treated by intravenous dye, colloidal copper, and colloidal lead with autopsy observations. Am. Med. Assoc., Jour. **93**: 1049–1056. 1929.

Ragle, H. E. ('29). Coccidioidal granuloma with report of a case. U. S. Naval Med. Bull. **27**: 657–661. 1929.

Rand, C. W. ('30). Coccidioidal granuloma: Report of two cases simulating tumor of spinal cord. Arch. Neurol. & Psych. **23**: 502–511. 1930.

Ricketts, H. T. ('01). Oidiomycosis (blastomycosis) of the skin and its fungi. Jour. Med. Res. **6**: 374–547. 1901.

Rixford, E., and T. C. Gilchrist ('96). Two cases of protozoan (coccidioidal) infection of the skin and other organs: I First case protozoan or coccidioidal pseudo tuberculosis, II A second case of protozoan infection. Johns Hopkins Hosp. Repts. **1**: 209–268. 1896.

Roblee, W. W. ('14). Report of a case of oidiomycosis. Calif. State Jour. Med. **12**: 387–389. 1914.

Ryfkogel, H. A. L. ('10). Coccidioidal meningitis: With secondary internal hydrocephalus and death (anaphylactic ?) following a second infection of Flexner's serum. Am. Med. Assoc., Jour. **55**: 1730–1732. 1910.

Salvat, P., et M. Fontoynont ('22). Contribution à l'étude des mycoses malgaches. Abcès sous-dermiques dus à l'Endomyces molardi (U. S.). Soc. Path. Exot., Bull. **15**: 311. 1922.

Seilin, J. ('19). Report of a case of dermatitis coccidiosa. Med. Rec. **95**: 360–361. 1919.

Stoddard, A. B., and E. C. Cutler ('16). Torula infection in man. Rockefeller Inst. Med. Res. Monogr. 6. 1916.

Stewart, R. A., and K. F. Meyer ('32). Isolation of Coccidioides immitis (Stiles) from the soil. Soc. Exp. Biol. & Med., Proc. **5**: 937–938. 1932.

Taylor, R. G. ('23). Coccidioidal granuloma. Am. Jour. Roentgenol. **10**: 551–558. 1923.

Tomlinson, C. C. ('28). Granuloma coccidioides. Med. Clin. North Amer. **12**: 457–462. 1928.

———, and Bancroft, P. ('28). Granuloma coccidioides. Am. Med. Assoc., Jour. **91**: 947–951. 1928.

Vuillemin, P. ('99). Les formes du champignon du muguet. Rev. Myc. **21**: 43. 1899.

———, ('10). Matériaux pour une classification rationelle des Fungi Imperfecti. Acad. Sci. Paris, Compt. Rend. **150**: 882. 1910.

Wernicke, R. ('92). Über einen Protozoenbefund bei Mycosis fungoides (?). Centralbl. f. Bakt. **12**: 859–861. 1892.

Wolbach, S. B. ('04). The life cycle of the organism of "Dermatitis Coccidioides." Jour. Med. Res. **13**: 53. 1904.

———, ('15). Recovery from coccidioidal granuloma. Boston Med. Surg. Jour. **172**: 94–96. 1915.

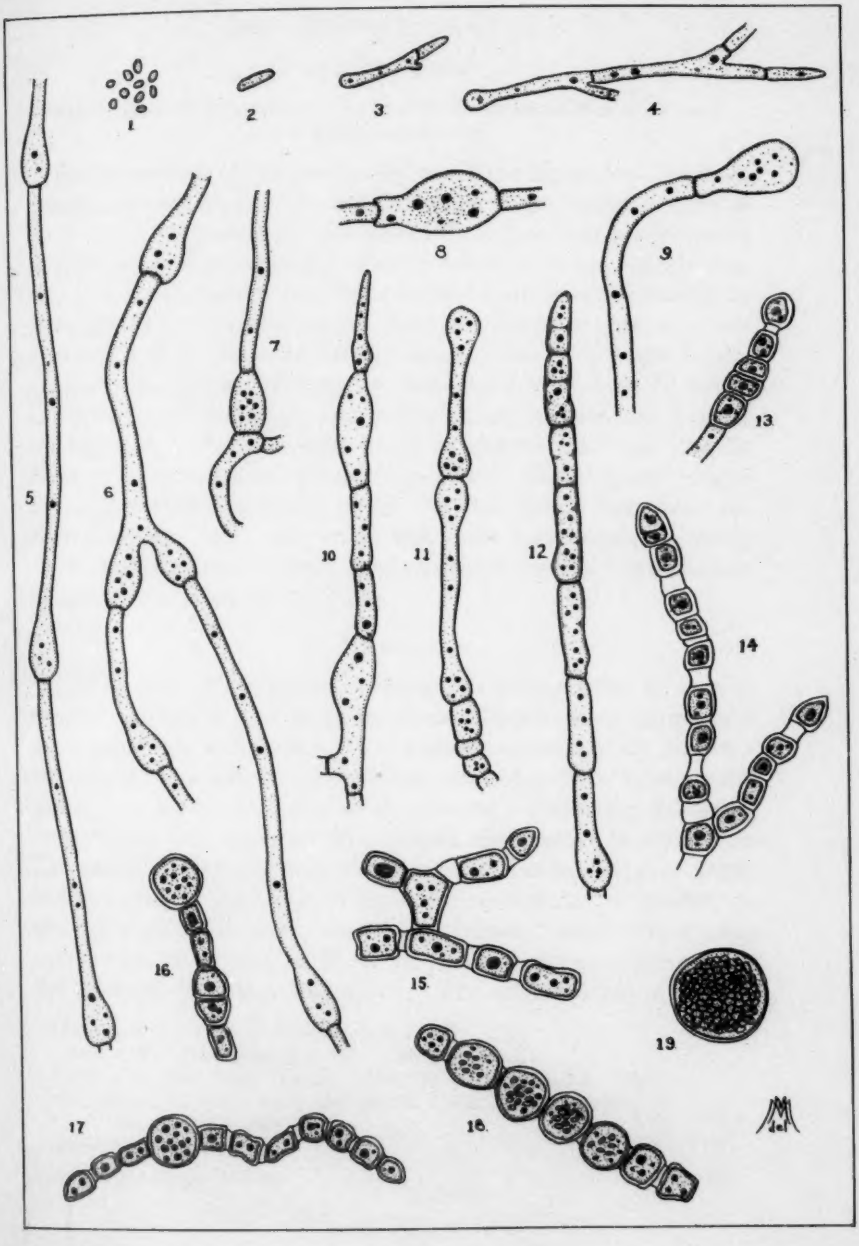
Zender, J. ('25). Sur la classification des Endomycétacées. Soc. Bot. Genève., Bull. **17**: 272–303. 1925.

Explanation of Plate

PLATE 25

Coccidioides immitis

Fig. 1. Young spore. × 960.
Fig. 2. Developing spore. × 960.
Figs. 3, 4. Young developing mycelium. × 960.
Fig. 5. Type of mycelium on nutrient agar. × 960.
Fig. 6. Type of mycelium on Sabouraud's agar. × 960.
Fig. 7. Portion of mycelium on glycerine agar. × 960.
Fig. 8. Probably a chlamydospore on Sabouraud's medium. × 1440.
Fig. 9. Terminal hypnospore. × 1440.
Fig. 10. Young hypha on glycerine agar. × 960.
Figs. 11, 12, 13, 14. Formation of arthrospores. × 960.
Fig. 15. Branching arthrospores. × 1440.
Figs. 16, 17. Old mycelium showing chlamydospores on malt extract agar. × 960.
Fig. 18. Formation of round chlamydospores from mycelium in anaerobic culture. × 960.
Fig. 19. Large, round ascogenous cell with endogenous spores. × 960.



MOORE—COCCIDIOIDES IMMITIS

THE GENUS DALDINIA

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The separation of the genus *Daldinia* from *Hypoxylon* has been a long and gradual process, one that even to-day appears to be of doubtful value to some mycologists. It is true that some species of *Hypoxylon* show some evidence of zonation in the entostroma, and it is these species that have caused part of the difficulty in determining generic limitations, since, for the most part, no other characters than zonation of the entostroma have been found. However, the genus *Daldinia*, as understood by the writer, seems to be worthy of retention, and in fact should be retained in order to segregate a characteristic group of species from the already large and cumbersome genus *Hypoxylon*. Since many species have been described in the genus *Daldinia* which have been accepted in part only, the writer feels that a monographic study of the genus, based on the careful examination of a large number of specimens, is highly desirable.

History

The history of the genus *Daldinia* has been similar to that of most of the larger and early recognized fungi,—very varied and with many ups and downs. As originally described by Scopoli,[1] the species now known as *Daldinia concentrica*, the type of the genus, was placed in *Valsa* as *V. tubersoa*. Following this early investigator was Hudson[2] who placed the species in the genus *Lycoperdon*, using the specific name *fraxineum*. A year later, Bolton[3] applied the name of *Sphaeria concentrica*. Schaeffer,[4] in the explanation of the plate in his 'Icones,' called the species *Lycoperdon atrum*, but later in the same work[5] gave the species the name of *Sphaeria concentrica*. The classification of Bolton

[1] Scopoli, J. A. Flora Carniolica, ed. 2, 2: 399. 1722.
[2] Hudson, W. Flora Anglica 2: 641. London, 1778.
[3] Bolton, J. Hist. Fung. Halifax 3: 180. *pl. 180* of appendix. 1789.
[4] Schaeffer, J. C. Icon. Fung. Bav. et Pal. 4: 131. *pl. 329*. 1800.
[5] *Ibid.* 129. *pl. 329*. 1800.

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was accepted by Fries[6] and others, undoubtedly because there was no such confusion of nomenclature as existed in the work of Schaeffer. An examination of the synonymy of *Daldinia concentrica*, as given later in this paper, will give ample evidence of the confusion that existed in the relation to the type, which was at that time the only species of *Daldinia*, hence little more need be said about the early conception of the species.

Prior to Persoon[7] the genus *Sphaeria* was a more or less heterogeneous group of species. In 1801 he divided the genus and placed *Sphaeria concentrica* Bolt. in his new section *Peripherícae* as species 11. Apparently this section was not recognized by Fries (*l.c.*), since in 1823 he placed the species in the tribe III, *Pulvinatae*, along with members of the subgenus *Hypoxylon*. At the same time he recognized two varieties of *Sphaeria* (*Hypoxylon*) *concentrica:* (b) *S. Eschscholzii* and (c) *S. obovata*. Later in 1849, Fries[8] revived Bulliard's genus *Hypoxylon* and divided that genus into three groups for which he proposed the names *Glebosae*, *Pulvinatae* and *Effusae*. *Hypoxylon concentricum* was placed in *Pulvinatae*. Meanwhile Léveillé[9] had created the tribe *Concentricae* in the genus *Sphaeria* to include, among others, his new species, *S. loculata* and *S. cingulata*, that were later assigned to *Daldinia*. In this division of *Sphaeria*, he was apparently supported by few mycologists of his time.

Daldinia, as it is understood to-day, was erected in 1863 by Cesati and DeNotaris,[10] who observed that the concentric zonation of the stroma was more pronounced in *Daldinia concentrica* than in other species of *Hypoxylon* with which the species had been formerly associated. The acceptance of the genus was not immediate, and, in fact, met with some opposition. Nitzschke,[11] in writing of *D. concentrica*, says "Die Trennung der vorliegenden und der ihr nächstverwandten, ausserdeutschen Arten von

[6] Fries, E. Systema Mycologicum **2**: 331. 1823.

[7] Persoon, C. H. Snyopsis methodica fungorum **1**: 8. *pl. 1, figs. 3–4.* Göttingen, 1801.

[8] Fries, E. Summa Veg. Scand., 383–384. 1849.

[9] Léveillé, J. H. Ann. Sci. Nat. Bot. III. **3**: 46–47. 1845.

[10] Cesati, V. & De Notaris, E. G. Schema Classif. d. Sferiacei **4**: 197–198. Gennaio, 1863.

[11] Nitzschke, T. Pyrenomycetes Germanici. 25–26. Breslau, 1867.

Hypoxylon und die Auffassung der Gruppe als besondere Gattung (*Daldinia Ces. et De Not.*), halte ich für nicht gerechtfertigt." This train of thought was also followed by Fuckel,[12] who recognized the species only as an *Hypoxylon* and admitted the existence of but one variety, namely *Hypoxylon concentricum* var. *obovatum*.

With the acceptance of *Daldinia* by Saccardo,[13] the objection to the genus appears to have lessened, although Cooke,[14] who made a study of *Xylaria* and *Hypoxylon*, wrote: "We should have preferred to have followed Nitzschke, and merged *Daldinia* (*Hypoxylon concentricum*) and *Bolinia* (*Hypoxylon tubulina*) in *Hypoxylon*. A comparison of the above characters will show that the only difference in *Daldinia* is the concentric stroma, although several of the globose *Hypoxyla* exhibit faint concentric zones when in good condition. The difference in *Bolinia* is that the perithecia are immersed, with rather long necks. These are very slight generic differences indeed as compared with some which might have been isolated, such as the very singular *Hypoxylon lycogaloides* B. & Br., and the equally strange and anomalous *H. solidum*, Schwz. If any species deserved to be raised to generic rank it was these." Saccardo's acceptance of the genus, then, marked the turning point, and from that date, separated on the conspicuously zonate character of the stroma, it has been accepted generally, even though a number of bridging species, such as *Hypoxylon Petersii*, *H. exsurgens*, *H. placentiforme*, and others, are known to exist.

Discussion of Diagnostic Characters

It has been observed by Theissen[15] that certain characters of the ascocarp vary or disappear with age, and thus alter one's conception of the species and lead to a certain amount of confusion in classification. He claims that the nature of the ostioles and the color of the ectostroma are functions of age and are not suitable for specific differentiation. This is in part true, but when such characters are correlated with others they may be very significant. Still, the changes that occur with advancing maturity

[12] Fuckel, L. Symbolae Mycologicae, Suppl. 2, p. 43. 1873.
[13] Saccardo, P. A. Sylloge Fungorum 1: 393. 1882.
[14] Cooke, M. C. Grevillea 11: 121–140. 1883.
[15] Theissen, F. Ann. Myc. 7: 3–5. 1909.

or subsequent weathering must be regarded in order to make a correct determination, since some closely related species are so similar in their youth that accurate determination cannot be made unless the investigator is familiar with all members of the genus.

The nature of the ectostroma may be used to a certain extent for the rough delimitation of certain species or groups of species. Thus the degree of laccateness, as in the case of *D. vernicosa*, which is laccate and shining, is somewhat specific. This is especially true when the species are studied in the field during their prime. If they are laccate they feel smooth, slippery, and somewhat moist, especially in moist or rainy weather when a somewhat viscous liquid abundant in the stroma appears to be exuded on the surface of the fruiting body. This character, however, should be used with caution, since species that are not laccate may be shiny black or bronze-black, but they, as a rule, become dull on weathering. Laccateness may be defined as a condition of the ectostroma which, as a result of the hardening of a viscous exudation, becomes varnished, has a brilliant sheen, and therefore simulates the appearance of a wet surface. Before the ascocarps reach maturity, the laccate nature of the surface may be obscured by the presence of a brownish or grayish lavender "bloom" of conidia.

The consistency of the ectostroma, while variable and somewhat difficult to define, may be fairly constant within a species. In some species it is very thin and brittle and weathers away soon after the fruiting body has reached maturity and has discharged its ascospores (pl. 29, fig. 1a); in others it is somewhat thicker and, while more resistant to weathering, is nevertheless easily punctured by the finger-nail; and yet others have a very durable outer layer.

Wrinkling of the surface of the fruiting bodies is at best an uncertain character and of little value if considered on its own merits. This is especially true in connection with such a species as *D. vernicosa*, of which the ascocarps when mature are filled with an abundance of a viscous liquid that disappears on drying and allows the inner tissue to collapse or to become loculate, with the accompanying wrinkling of the outer surface. The pro-

portion of liquid to tissue varies not only with the age of the fruiting body but also with the weather, since during seasons of continuous rain growth is more rapid, the liquid is more abundant, and the hyphal elements appear to deliquesce more rapidly. Similarly, folding, lobing, and other gross characters appear to be influenced greatly by the nature of the substratum and by environmental conditions. Thus when growing on such porous wood as that of *Betula*, *D. concentrica* attains a larger size than it does when inhabiting a more dense substratum. As will be noted later in the discussion under *D. vernicosa*, the nature of the substratum and the environmental conditions may be an explanation of the huge fruiting bodies of the species described as *D. fissa* by Lloyd.[16] In brief, then, external characters should not be used too freely because of the pronounced effects of environmental conditions.

By means of the entostroma, the species of *Daldinia* may be divided into two easily recognized groups:—one characterized by having a dense fibrous context which is usually brown or shades of brown or brownish-gray and marked with relatively thick, darker brown, concentric zones; the other by having collapsing, white or grayish, loculate zones alternating with relatively thin darker zones. The loculate character of the stroma of the latter group appears to be the result of the loss of the abundant viscous fluid which may have been formed by the gelatinization of a portion of the hyphal elements. The darker zones, according to Miller,[17] are formed by the disintegration of perithecial initials, followed by continued growth beyond the perithecial initials to produce successive zones, the number of zones being limited by the time of formation of the perithecia. Zonation and the color and nature of the entostroma are sometimes very valuable as specific characters.

The amount of protrusion of the ostioles, while somewhat variable in some species, appears to be a valid and easily recognizable character in others. For example, *D. cuprea* and *D. clavata* are readily separated on this character alone (pl. 30, figs. 6, 7). Fortunately, there are other characters that are correlated

[16] Lloyd, G. C. Myc. Notes **7**: 1313. *pl. 306, fig. 2986.* 1929.
[17] Miller, J. H. Mycologia **20**: 328. 1928.

with this. In *D. concentrica* this character, while varying within a rather narrow range, may also be used to some extent; however, it should not be relied upon too greatly, since, when the surface is made minutely papillate by the protrusion of the ostioles, the fruiting body has a somewhat different aspect. These same words also apply to *D. Eschscholzii*, in which, as shown in pl. 29, fig. 5, the ostioles are merely punctate, whereas in other specimens (pl. 29, figs. 3, 6) they may be minutely papillate. The amount of protrusion of the ostioles, in some instances, appears to be dependent on the amount of humidity. As is shown in pl. 28, figs. 7, 8, the ostioles on the upper surface of the ascocarp are represented by papillate protrusions, while those on the lower surface of the same fruiting body, closer to the surface of the substratum, are not only papillate but *Rosellinia*-like. With such variations and exceptions in mind, the degree of prominence and the distance between the ostioles may prove a distinct aid in recognizing the species.

The size and shape of the perithecia may be somewhat variable, partly because of environmental influences, but when correlated with other characters they are sufficiently constant to be of diagnostic value (pls. 31–33). In the majority of species the perithecia are arranged in a single row around the periphery of the stroma, that is, the perithecia are monostichous; but in a few, the perithecia are polystichous and arranged either evenly or unevenly in two rows. It is possible that the polystichous arrangement may be due to the crowding of the perithecia which forced them to take positions at unequal levels; or it may be a temporary condition that has resulted from the sudden checking of growth. In any case, the polystichous arrangement often varies and may exist in one part of an ascocarp whereas in another the perithecia may be monostichous.

It is important when studying the perithecia, to cut the sections in a plane that passes longitudinally through the perithecia as close to the ostiole as possible. By this procedure errors in observing the size and shape of the perithecia will be minimized. Also, in order to determine the true nature of the ostioles, this precaution should be observed. Miller[18] makes the statement:

[18] Miller, J. H. *l. c.* 329.

"The perithecial necks do not penetrate through the ectostroma, so the ostiola are umbilicate." The writer, after a study of many sections, has come to the conclusion that all of the necks of mature perithecia do penetrate the ectostroma and at least reach the surface of the fruiting body, as is shown in pl. 31, fig. 1, pl. 32, figs. 1, 2, 4, pl. 33, figs. 1, 3, 5.

The ascospores of members of this genus are predominantly purple-black in mass, but when mounted and observed under the microscope they vary, according to the species, from amber color to brown or brownish-black. As is characteristic of most members of the Xylariaceae, the spores are provided with a longitudinal cleft through which the germ-tube subsequently grows. In certain species, such as *D. cuprea* and *D. albozonata*, the amber-colored spores have a dark brown mesospore, whereas the dark spores have an amber-colored one. The exospore is hyaline and takes the blue stain of the lactophenol-cotton-blue mounting medium. This outer layer sloughs off during the process of spore germination.

While there is no great variety of shapes among the ascospores of the different species, such differences as do exist vary but little within a single species and so may be used as taxonomic characters. Usually the spores are ellipsoid and symmetrical, but they may also be inequilaterally ellipsoid with either acutely or bluntly rounded ends (pls. 26 and 27). Again, these ends may be hyaline and refractive, but the character does not appear to be one on which too great reliance should be placed.

There is no specifically characteristic method of ascospore discharge, for in all species with which the writer is familiar in the field, the spores are ejected in long threads that soon break up. Theissen[19] has also made this observation, and Rick[20] described *D. barbata* as a new species because of the fact that the ascospores were extruded in such a manner.

[19] Theissen, F. *l. c.* 3.
[20] Rick, J. Broteria 5: 50. 1906.

Geographical Distribution

In a previous paper, the writer[21] has had occasion to remark on some of the factors that appear to be of importance in limiting the number and distribution of certain species. It is realized that by no means have all factors been considered, nor is it possible in this paper to add any further information that is based on experimental data.

Of the thirteen species recognized in this paper, but four have a relatively limited distribution, that is, they are confined to one continent or a portion thereof. Thus *Daldinia simulans*, as will be seen from an examination of the accompanying maps, is confined to the United States and is apparently not found outside of the Mississippi Valley where it has been found only in Ohio, Illinois, and Missouri. *D. clavata*, apparently a rare species, has been collected in North and South America, ranging from Mexico to Argentina. There are no doubts as to the distribution of *D. albozonata*, for this species has only been collected in Africa, being found in French Equatorial Africa (formerly Kamerun), Angola, and in Uganda. There is the possibility that the range of the species is coextensive with that of the West African rain forest. The only representative of the genus that is confined to Europe is *D. caldariorum* which is known only from the original collection that was made in a fern house in the Botanical Garden at Berlin. What significance this species might have is difficult to determine, for it cannot be said, without fear of contradiction, that the species is endemic to Europe. From the very nature of the place in which it was collected there is the possibility that the spores may have been introduced from almost any country. It has in fact been reported from Brazil.

Daldinia concentrica and *D. vernicosa* both have a very wide range of distribution, but with a few exceptions the two species appear to be confined to the regions outside of areas included between the 70° F. isotherm lines, or north of 30° north latitude. Within the boundaries of North America as thus delimited, it is obvious that the distribution has some particular climatic and humidity relation, since the species studied were collected either in river valleys or near large bodies of water, regions in which

[21] Child, M. Ann. Mo. Bot. Gard. 16: 411–486. 1929.

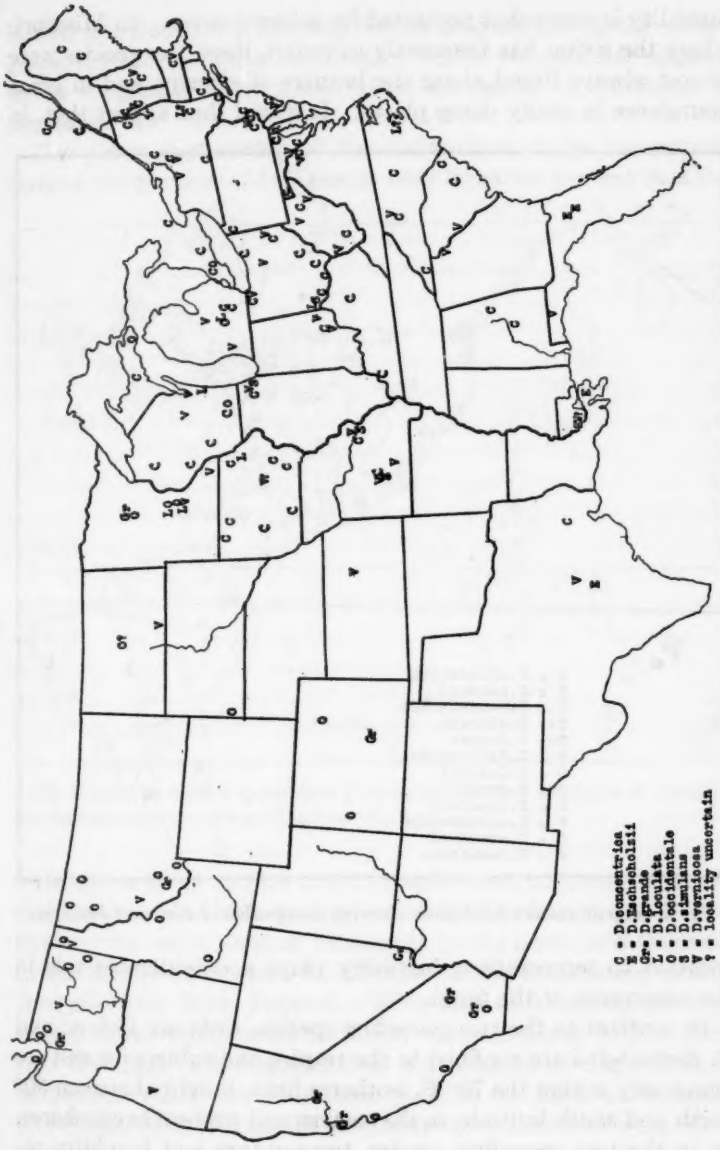

Fig. 1. Map of United States showing distribution of species of *Daldinia*.

humidity is somewhat protected by a forest cover. In Missouri, where the writer has frequently collected, these two species were almost always found along the borders of streams and in great abundance in shady damp places. It would thus appear that, in

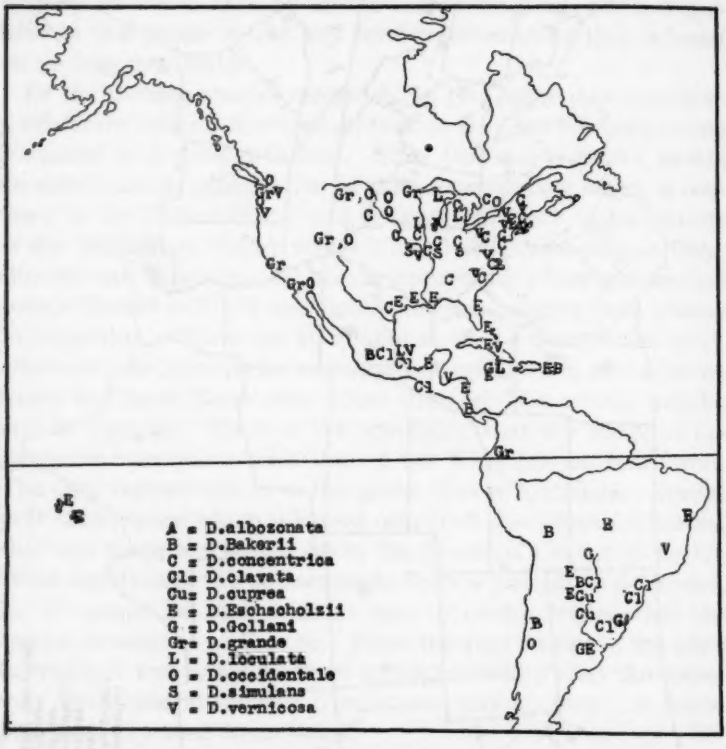

Fig. 2. Map of western hemisphere showing distribution of species of *Daldinia*.

addition to temperature, humidity plays a considerable role in the occurrence of the fungi.

In contrast to the two preceding species, *Daldinia Bakerii* and *D. Eschscholzii* are confined to the tropics and subtropics and are found only within the 70° F. isotherm lines, roughly between 30° north and south latitude, in the eastern and western hemispheres. As in the two preceding species, temperature and humidity re-

lations apparently are limiting factors in determining the occurrence of these two fungi, although a higher temperature appears to be of greater importance when the factor of humidity is favorable.

The above explanation of the distribution of the representatives of the genus would be simple were it not for the fact that the

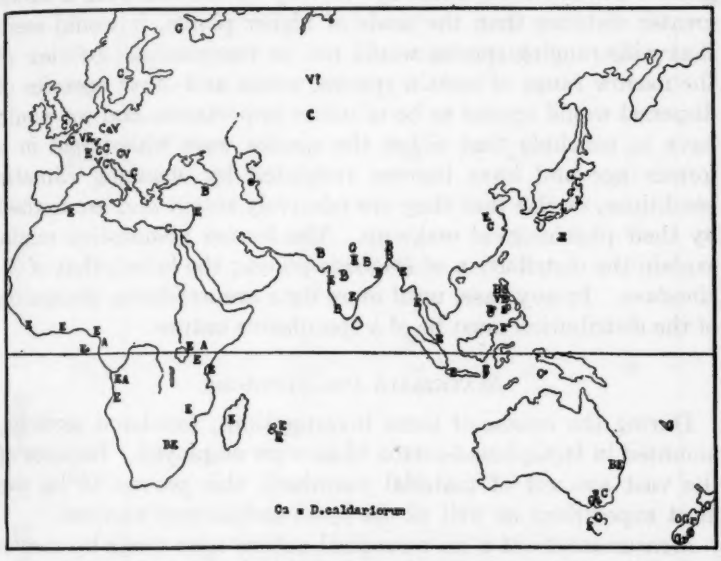

Fig. 3. Map of eastern hemisphere showing distribution of species of *Daldinia*. For explanation of symbols see figs. 1 and 2.

distribution of all species is not in agreement with such a simple explanation. *Daldinia grande*, which in North America is confined to the states west of Minnesota in the north and Colorado in the south, also occurs in Ecuador in South America, and in Australia and New Zealand. With such contrasting seasonal ranges as are represented in North America alone, it is evident that the range of the species does not coincide with any definite isothermal zone, yet if it were possible to have an intimate knowledge of the localities in which the collections were made there might possibly be some modifying circumstances that would

prove the distribution to be in general agreement with the theory suggested above.

In the present state of our knowledge, it is very difficult to find much rhyme or reason for the distribution of fungi, partially, it is true, because of the relatively few collections that have been made as compared with the number of collections of phanerogams. Furthermore, since spores may be carried over a much greater distance than the seeds of higher plants, it would seem that wide-ranging species would not be uncommon. In view of the narrow range of certain species, winds and other agencies of dispersal would appear to be of minor importance, and we would have to conclude that either the species were widespread in a former age and have become restricted by changing climatic conditions, or else that they are relatively recent and are limited by their physiological make-up. The former assumption might explain the distribution of *Daldinia grande;* the latter, that of *D. simulans*. In any case, until more data are available, discussion of the distribution must be of a speculative nature.

Materials and Methods

During the course of these investigations, free-hand sections, mounted in lactophenol-cotton blue, were employed. Because of the vast amount of material examined, this proved to be the most expeditious as well as the most satisfactory method.

Measurements of a microscopical nature were made by means of a calibrated eye-piece micrometer. It was at first thought that, in order to obtain a fair representation of the range in spore size, a large number of measurements was necessary, and accordingly one hundred spores from each ascocarp were measured. However, after comparing the count of one hundred spores with that of twenty-five, it was found that the results when plotted graphically were practically identical, and it was therefore considered unnecessary to measure more than twenty-five spores per ascocarp, especially when that number from each of several ascocarps of the same collection were measured. The results showed that there was very little variation since the spores fell within the same size-class or size-classes. Within limits, the same statement may be made in regard to the measurements of ascospores of

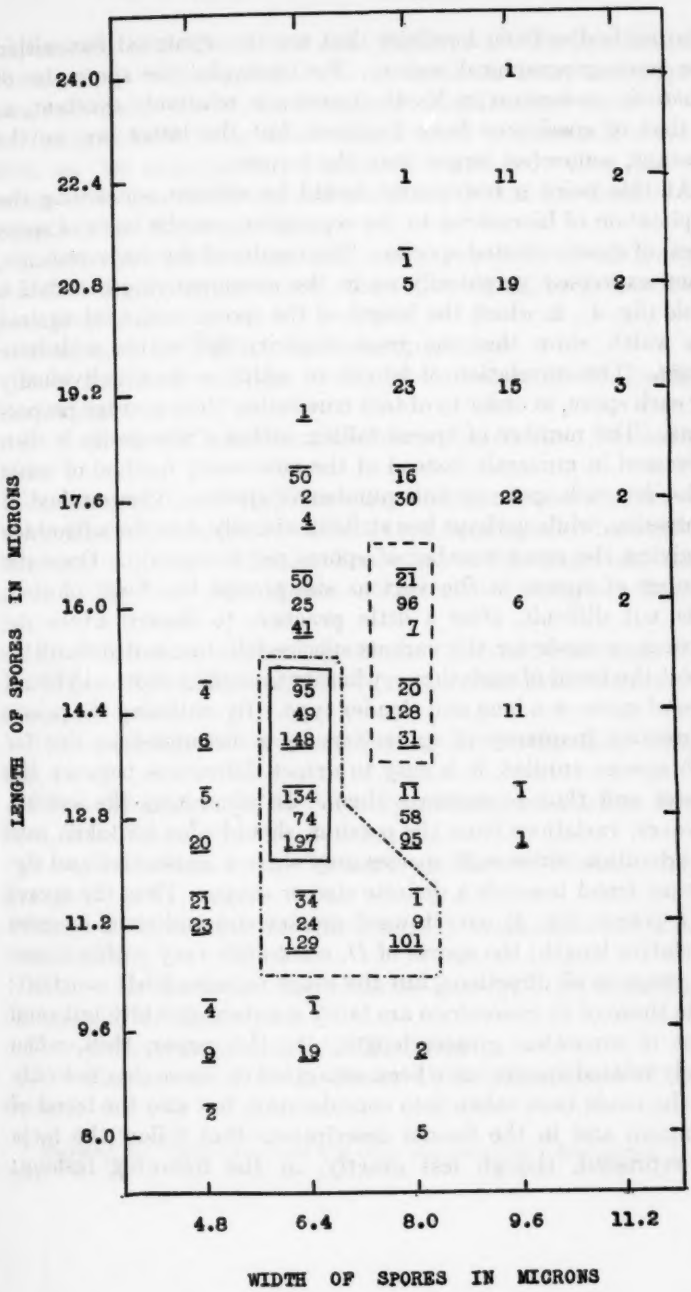

Fig. 4. Correlation table expressing application of biometrics to separation of closely related species. *D. concentrica* = $\overline{0}$ = .-.-.-.-; *D. grande* = 0 = - - - - - - -; *D. occidentale* = $\underline{0}$ = ———.

fruiting bodies from localities that are far separated but within the same geographical region. For example, the spore size of *Daldinia concentrica* in North America is relatively constant, as is that of specimens from England, but the latter are, on the average, somewhat larger than the former.

At this point a few words should be written concerning the application of biometrics to the separation, on the basis of spore sizes, of closely related species. The results of the measurements, when expressed graphically as in the accompanying correlation table (fig. 4), in which the length of the spores is plotted against the width, show that the great majority fall within a definite range. The correlation of length to width is done individually for each spore, in order to obtain true rather than average proportions. The number of spores falling within a size-group is then expressed in numerals instead of the customary method of using a dot for each spore or unit number of spores. This method of expression, while perhaps less striking visually, has the advantage of giving the exact number of spores per size-group. Once the number of spores in the various size-groups has been plotted, it is not difficult, after a little practice, to discern where the maxima or mode for the various species fall, nor is it difficult to detect the trend of variation,—whether towards a short and broad type of spore or a long and slender type. By outlining the points of greatest frequency of spore sizes by a distinguishing line for each species studied, it is easy to detect differences between the species and thus to separate them. In separating the species, however, variations from the maxima should also be taken into consideration, since each species may show a distinctive and significant trend towards a definite size or shape. Thus the spores of *D. grande* (fig. 4) vary toward greater size and some increase in relative length; the spores of *D. occidentale* vary within a narrow range in all directions, but the shape remains fairly constant; while those of *D. concentrica* are fairly constant in width but tend to be of somewhat greater length. In this paper, then, when closely related species have been separated on spore size, not only has the mode been taken into consideration but also the trend of variation, and in the formal descriptions that follow the facts are expressed, though less exactly, in the following fashion:

"spores (8)–14.4–16–(27.2) x (6.4)–8–(11.2) μ." In this formula, the numerals within parentheses represent the extremes of spore size, and those without, the average or most frequent sizes.

Measurements of the ascocarps are given in three sets of numbers, as, for example, 2.5–3 x 2–3 x 1–2.5 cm. The first set refers to the length, the second to the width, and the third to the height of the fruiting bodies.

Colors when within quotation marks are those of Ridgway.[22]

In the citation of specimens, the following abbreviations have been employed:

A = Museo de la Plata, La Plata, Argentina.

B = Herbarium of the Botanical Museum, Berlin-Dahlem, Germany.

F = Farlow Herbarium, Harvard University, Cambridge, Mass.

Ia = Herbarium of the University of Iowa, Iowa City, Ia.

L = Herbarium of the late C. G. Lloyd, Smithsonian Institution, Washington, D. C. The numbers cited in this paper are those given by the U. S. Department of Agriculture to replace those of Lloyd.

Li = Herbarium of David H. Linder, Harvard University.

MBG = Herbarium of the Missouri Botanical Garden, St. Louis, Mo.

NC = Herbarium of University of North Carolina, Chapel Hill, N. C.

NY = Herbarium of the New York Botanical Garden, Bronx Park, New York City.

P = Herbarium of the Museum of Natural History, Paris, France.

Pa = Herbarium of the Academy of Natural Sciences, Philadelphia, Pa.

PDS = Herbarium of Plant Disease Survey, U. S. Department of Agriculture, Washington, D. C.

Po = Herbarium of Alfred H. W. Povah.

S = Herbarium of C. L. Shear, U. S. Department of Agriculture.

[22] Ridgway, R. Color standards and color nomenclature. Washington, D. C., 1912.

Sh = Herbarium of Paul Shope, Boulder, Colorado.
St = Herbarium of the State Museum, Stockholm, Sweden.
T = Herbarium of the University of Texas.
W = Herbarium of Leva B. Walker, Lincoln, Nebraska.

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Taxonomy

DALDINIA

Stromata superficial, hemispherical, subglobose, globose, turbinate, or clavate, sessile, substipitate or stipitate. Ectostroma carbonaceous, at first pruinose, later dull black, shining, or laccate. Entostroma dense or lacunate, white, gray, or brown, *conspicuously concentrically zonate*. Perithecia claviform to subglobose, immersed in the stroma, not umbilicate with obsolete to protruding or papillate ostioles. Asci cylindrical, soon disappearing, 8-spored, the spores obliquely uniseriate. Ascospores simple, ellipsoid to navicular, light to dark brown, translucent or opaque.

The genus as here understood is separated from *Hypoxylon* by its conspicuously zonate stroma. In some species of *Hypoxylon*, however, there may be slight indications of zonation, but these have been excluded from the genus. Usually the ascocarp of *Hypoxylon* varies from resupinate to sessile; rarely are they substipitate, and never stipitate. Also, while tropical forms of *Hypoxylon* may attain enormous sizes and simulate *Daldinia* externally, the ectostroma is usually very much roughened, very thick, and the entostroma very dense and homogeneous or woody.

KEY TO THE SPECIES OF DALDINIA

1. Inner context dark brown or brownish, persistent, dense. (See also *D. Bakerii*) 2
1. Inner context light, lighter zones white or some shade of gray, dense or collapsing, with persistent dark zonal lines 6

2. Ascocarp sessile or substipitate, stipe when present broad, exceeding 5 mm. in thickness and not cylindrical....3
2. Ascocarp definitely stipitate, the stipe cylindrical, less than 5 mm. thick and supporting a globose to subglobose fertile head....*8. D. loculata*
3. Ostioles obsolete to punctiform; ascocarp smooth or very slightly papillate; spores (8)–11.2–(14.4) x 4.8–(6.4) μ....*4. D. Eschscholzii*
3. Ostioles prominent, papillate to mammiform; ascocarp somewhat rough to rough....4
4. Ascospores (8)–14.4–16–(27.2) x 6.4–8.–(11.2) μ; ascocarps usually large, irregular, always sessile; perithecia mostly polystichous....*3. D. grande*
4. Ascospores smaller; ascocarps not as above....5
5. Mature ascocarps bronze-black; spores bluntly rounded, (9.6)–12.8–(19.2) x (4.8)–6.4–8–(9.6) μ....*2. D. occidentale*
5. Mature ascocarps vinaceous-black to dull black; spores rounded, tapering at either end, (8)–12.8–14.4–(20.8) x (4.8)–6.4–(8) μ....*1. D. concentrica*
6. Inner context dense, not collapsing....7
6. Inner context loose, collapsing....9
7. Ostioles prominent; ascospores (9.6)–14.4–(24) x (4.8)–6.4–(11.2) μ..*5. D. Bakerii*
7. Ostioles obsolete to punctiform....8
8. Perithecia subspherical; ascospores (6.4)–9.6–(12.8) x (3.2)–4.8–(6.4) μ. ....*6. D. Gollani*
8. Perithecia elongate-ovoid; ascospores (9.6)–11.2–(14.4) x 4.8–(6.4) μ.... ....*7. D. caldariorum*
9. Ascocarps turbinate or irregularly turbinate....10
9. Ascocarps clavate....12
10. Spores small, (6.4)–8–(9.6) x (2.4)–3.2–(4.8) μ....*11. D. albozonata*
10. Spores larger....11
11. Ascocarp vinaceous, laccate at maturity; stipe inconspicuously zonate; spores (8)–11.2–12.8–(17.4) x (4.8)–6.4–(8) μ....*9. D. vernicosa*
11. Ascocarp drab or drab-brown; stipe zonate; spores (9.6)–11.2–(14.4) x (4.8)–6.4–(8) μ....*10. D. simulans*
12. Perithecia confined to the upper part of the stipe-like stroma, with conspicuous ostioles; stipe externally zonate with annulate thickening...*12. D. cuprea*
12. Perithecia over entire stroma, not confined to upper half; ostioles obsolete to punctiform....*13. D. clavata*

1. Daldinia concentrica (Bolt.) Cesati & DeNotaris, Schema Classif. d. Sferiacei 4: 197. 1863.

Fungus fraxineum etc. Raius, Hist. Pl. 1: 109. 1686; Syn. Stirp. Brit. ed. 3. 16. No. 20. 1724.

Valsa tuberosa Scopoli, Fl. Carniolica, ed. 2. 2: 399. 1722.

Lycoperdon fraxineum Hudson, Fl. Angl. 2: 641. 1778.

Sphaeria concentrica Bolton, Hist. Fung. Halifax 3: 180. *pl. 180* of appendix. 1789.

Sphaeria tunicata Tode, Fung. Meckl. Sel. **2**: 59. *pl. 17, fig. 130 a–g.* 1791.

Lycoperdon atrum Schaeffer, Icon. Fung. Bav. et Pal. **4**: 131. *pl. 329.* 1800 (as *Sphaeria concentrica* p. 129).

Sphaeria fraxinea Withering, Arrang. Brit. Pl. ed. 5. **4**: 429. 1812.

Sphaeria hemisphaericae Nees, Syst. d. Pilze **1**: 291. 1817.

Sphaeria concentrica Bolton ex Fries, Syst. Myc. **2**: 331. 1823.

Stromatosphaeria concentrica Greville, Fl. Edinensis, 355. 1824.

Sphaeria (*Pulvinata*) *placenta* Link, Linnaea **5**: 539–540. 1830.

Hemisphaeria concentrica Klotzsch, Acad. Caes. Leop. Nova Acta **19**: 241. 1843.

Pl. 27, fig. 4; pl. 28, fig. 4; pl. 29, figs. 1–2, 4, 7, 9; pl. 30, fig. 1; pl. 33, fig. 1.

Stromata hemispherical to globose, sessile to stipitate, single or coalescing, 1.1–10 x 1–7 x 1–7 cm., rubiginous when young, erumpent from the bark or superficial on decorticated wood. Ectostroma moderately thin, "Dark Vinaceous Brown," "Sorghum Brown," "Hay's Brown," "Drab," "Army Brown," or more frequently "Vinaceous Brown," later becoming black and either dull or shiny. Entostroma densely fibrous, persistent, conspicuously concentrically zoned, the lighter "Dilute Snuff Brown," "Fuscous," "Hair Brown," "Benzo Brown," or "Deep Mouse Gray" zones up to three times wider than the darker "Bone Brown" zones. Perithecia usually monostichous, rarely polystichous, claviform to subclaviform, 518–1776 x 185–592 μ, the walls 14.8–37 μ thick. Ostioles punctiform to somewhat prominent. Ascospores inequilaterally ellipsoid, brown, the ends sometimes of a lighter color and more refractive, (8)–12.8–14.4–(20.8) x (4.8)–6.4–(8) μ. Conidia ellipsoid, hyaline, 2.4–3.2 x 4.8–6.4 μ.

On wood of a wide variety of deciduous trees. Cosmopolitan.

Bolton described this species in 1789, and since that time it has

been glorified and belittled by various descriptions and synonyms. Persoon[23] described the variety *pedicellata* of *Sphaeria concentrica*, but in his figure 4 the longitudinal section of the fruiting body clearly shows the entostroma to be white, and, together with the characters of the fruiting body as a whole, shows that the variety should be considered as synonymous with *D. vernicosa*. In referring to the figures in the text (p. 8, β), Persoon refers to the variety *pedicellata* of the plate as variety *stipitata*. Variety *obovata* is also ascribed to this writer by Fries,[24] but this is an obvious misunderstanding which apparently resulted from the fact that the first word of an indented line was "obovata." Fries also linked Nees with this error, and in 1882 Saccardo[25] also ascribed the variety to Nees and mentions that it is close to *D. vernicosa*.

Fries[26] makes *Hypoxylon durissimum* (Schw.) Berk. a synonym of *Sphaeria concentrica*, but Cooke[27] says that the species should be considered synonymous with *Hypoxylon marginatum*. A photograph of the exterior and a preparation of ascospores from the type have been studied by the writer who is inclined to follow Cooke's belief, at least as far as the generic status is concerned.

The fruiting bodies of this species are fairly constant in their macroscopical appearance, but exceptionally, when the species grows on soft porous wood such as birch, they attain a greater size. The ascospores of these large forms, however, are in agreement with those of the typical material. As has been previously noted (p. 442) the spores from European material tend to be somewhat larger than those of typical American material, although they are in general agreement, and hence it seems to the writer that the creation of a variety is scarcely warranted.

Specimens examined:

Exsiccati: Allescher & Schnabel, Fungi Bavarici, 76; Bartholomew, Fungi Columb., 2013; Cooke, Fungi Brit. Exsicc. I, 669; Cooke, Fungi Brit. Exsicc. II, 216; de Thümen, Myc. Univ., 10883; Fries, Scleromyceti Sueciae, 141; Karsten, Fungi Fennici, 265; Linhart, Fungi Hungarici, 261; Plowright, Sphaer.

[23] Persoon, C. H. Syn. Meth. Fung. 1: 8. *pl. 1, figs. 3–4.* 1801.
[24] Fries, E. *l. c.* 1823.
[25] Saccardo, P. A. Syll. Fung. 1: 393. 1882.
[26] Fries, E. Nov. Symb. Myc. 114. 1851.
[27] Cooke, M. C. Grevillea 11: 131. 1883.

Brit., 17; Ravenel, Fungi Caroliniani, Fasc. III, 49; Roumeguére, Fungi Gall. Exsicc., 3946; Torrend, Fungi Sel. Exsicc., 142.

CANADA: Quebec, Lake St. John, Aug. 1910, *Isabel M. Walker* (L 10908); *G. Ducharne* (L 12300); Ontario, *Isabel M. Walker* (L 10915); London, on decaying wood, July 1904, *John Dearness, 2013* (F); Toronto, July 1908, *Thos. Langton* (L 10926).

UNITED STATES:

Maine: Milo, on alder, Aug. 18, 1920, *C. L. Shear* (S 5582); Mt. Katahdin Trail, on *Fagus* log, Aug. 21, 1920 (S); *C. C. Hanner* (L 11055).

New Hampshire: Hanover, on *Alnus*, Sept. 30, 1902, *A. H. Chivers* (F); Grantham, *Kate Jones* (L 10886, 12301); Hebron, on *Ulmus* post, *Mrs. R. D. Randlett* (L 10660).

Vermont: Middlebury, Oct. 1910, *C. G. Lloyd* (L 10918); Pawlett, on *Betula papyrifera*, April 17, 1925, *D. H. Linder, 598* (Li); Winooski, *D. B. Griffin* (L 10939).

Massachusetts: Waltham, Sept. 7, 1913, *G. E. Morris* (L 10916).

Connecticut: Morris, Aug. 1915, *B. O. Dodge* (PDS); without locality, *T. H. MacBride* (Ia 1345).

New York: Adirondacks, *F. H. Ames* (L 10874), *E. Knäbel* (L 10818, 10903); Berne, on maple, Aug. 31, 1925, *C. L. Shear* (S); Brooklyn, Oct. 24, 1914, *F. H. Ames* (L 10873, 10906); Buffalo, *G. W. Clinton* (F, PDS); Long Island, *G. C. Fisher* (L 10921); Orient, on *Quercus*, Aug. 19, 1921, *Roy Latham* (L 12177); Ithaca, on *Hamamelis virginiana*, April 30, 1893, *Atkinson* (MBG 15977), Oct. 2, 1910, *F. A. Wolf, 60* (PDS); July 10, 1901, *C. O. Smith* (PDS); Syracuse, *L. H. Pennington* (L 10819); Watkins, on *Ulmus* stumps, July 2, 1892, *D. G. Fairchild* (PDS).

New Jersey: Newfield, *J. B. Ellis* (PDS); Trenton, *E. B. Sterling* (L 10922).

Pennsylvania: Philadelphia, *Mrs. Hannah Streeter* (L 10917, 10933); Mauch Chunk, Sept. 13, 1914, *C. J. Humphrey* (L 10808); without locality, *Eliz. C. Cox* (L 10825).

Maryland: Analostan Island, on rotten *Celtis*, Sept. 14, 1919, *C. L. Shear* (S); Baltimore, *Chas. C. Plett* (L 10931); Grand Falls, April 19, 1925, *F. T. Eagan* (PDS); High Island, on

Betula nigra, July 29, 1922, *A. E. Jenkins* (PDS); Island Park, Sept. 16, 1918, *C. L. Shear* (S), on wild *Vitis*, Sept. 16, 1918, *C. L. Shear* (S); Leitersburg, *W. T. Lakin* (L 10884); Mid River Island, on bark, July 4, 1924, *F. T. Eagan* (PDS 66346); North Beach, June 11, 1922, *E. K. Cash* (PDS); Oakland, Sept. 17, 1917, *C. L. Shear* (S), on *Crataegus*, Sept. 17, 1917, *C. L. Shear* (S 5583), on young and old maple logs, Sept. 17, 1917, *C. L. Shear* (S); Oxen Run, on *Carpinus*, Oct. 21, 1921, *J. R. Weir* (PDS); Plummers Island, on *Acer*, Sept. 28, 1919, *C. L. Shear* (S); Sycamore Island, on *Rhus*, May 15, 1927, *A. E. Jenkins* (PDS).

District of Columbia: Rock Creek Park, on *Platanus occidentalis*, Sept. 24, 1916, *H. R. Rosen & E. H. Siegler* (PDS), *E. Knäbel* (L 10935), on *Quercus alba*, June, 1912, *J. R. Weir*, *7462* (PDS).

Virginia: Arlington Farm, Dec. 1916, *C. L. Shear* (S); Black Pond, Aug. 13, 1922, *E. K. Cash* (PDS); Clarendon, on *Acer rubrum*, April 6, 1923, *W. W. Diehl & J. R. Weir* (PDS), on *Acer* ?, March 11, 1923, *J. R. Weir* (PDS), on *Acer rubrum*, Jan. 3, 1926, *J. R. Weir* (PDS), on *Diospyros virginiana*, Oct. 10, 1926, *J. R. Weir* (PDS 66660); Dead Run, Fairfax Co., Sept. 30, 1923, *E. K. Cash* (PDS); Great Falls, on *Acer saccharinum*, Aug. 12, 1926, *J. R. Weir* (S), on *Acer Negundo*, Oct. 30, 1925, *F. T. Eagan*, *441* (PDS); without locality, apple orchard, *A. M. Beckwith* (PDS).

North Carolina: Canton, *William Holden* (L 10909); Chapel Hill, on dead birch, fall of 1928, *Andrews* (NC 8259), on bark of deciduous trees, Sept. 1922, *W. C. Coker* (NC 5399); Winston Salem, on decaying stump, Aug. 1922, *Dr. Shallert* (NC 8555); Waynesville, on osage orange, *Mary Fitzgerald* (L 10940, 10943).

South Carolina: Pisgah, on *Betula lutea*, July 16, 1910, *A. H. Graves* (L 11877); Pee Dee River, Nov. 7, 1920, *C. L. Shear* (S).

Alabama: Auburn, on willow, Nov. 25, 1897, *F. S. Earle* (MBG 16365), 1896, *Underwood* (Ia); Montgomery, *R. P. Burke* (L 10907).

Ohio: Adams County, May 4, 1930, *A. H. W. Povah* (Po);

Barnesville, *Emma E. Laughlin* (L 10885); Cincinnati, July 1884, *Dr. Byrones* (PDS), Oct. 2, 1919, *H. Cugner* (PDS 6581), Sept. 8, 1920, *C. G. Lloyd* (L 11765); Linwood, Aug. 26, 1920, *C. G. Lloyd* (L 10820); Mineral Springs, May 4, 1930, *A. H. W. Povah* (Po 3); Norwood, July 1897, *C. G. Lloyd* (L 10945); Oberlin, *Emma J. Carl* (L 10924); Oxford, small logs, Oct. 12, 1920, *B. Fink, 1048* (PDS, F); Salem, *B. Leeper* (L 10864); Toledo, *W. R. Lowater* (L 11794), *Mrs. Albert J. Wolfert* (L 10888).

West Virginia: Cooper's Rock, on *Betula lenta* (?), July 28, 1907, *C. L. Shear, 3048* (PDS); Eglon, Sept. 5–10, 1915, *C. G. Lloyd* (L 10914); Fairmont, *Rev. A. Boutlou* (L 10811, 10812, 10835, 10853, 10887); Fayette Co., April 15, 1893, *L. W. Nuttall, 905* (PDS).

Michigan: Ann Arbor, on *Acer rubrum*, Sept. 10, 1894, *L. N. Johnson* (Po 229a), on *Betula* logs, July 1914, *E. B. Mains* (PDS 12388); East Lansing, *E. A. Bessey* (L 10822); Rock River, on *Alnus*, Aug. 24, 1927, *A. H. W. Povah* (Po 229d).

Indiana: Scottsburg, 1907, *J. R. Weir, 8964* (PDS), on *Hicoria ovata*, May 1901, and June 1912, *J. R. Weir, 2751, 7433* (PDS).

Kentucky: Crittenden, Oct. 5, 1914, *C. G. Lloyd* (L 10937); Lexington, *H. Garman* (L 10905).

Wisconsin: Dells, June 1927, *A. H. W. Povah* (Po 1); Hayward, Sept. 30, 1919, *C. J. Humphrey* (L 10810); Madison, on *Acer*, Aug. 9, 1920, *E. E. Hubert* (L 10807); River Falls, Oct. 1887, *F. H. King* (MBG), Oct. 1887, *Wm. Trelease, 2* (MBG).

Illinois: Evanston, Sept. 29, 1928, *H. Fox* (Po 229); without locality, *MacDougal* (L 10852).

Iowa: Decorah, on logs, Sept. 5, 1885, *E. W. D. Holway* (PDS); Decatur Co., Nov. 6, 1900, *R. L. Smith* (Ia 1350); Fairfield, *J. F. Clarke* (L 10934); Fort Dodge, *O. M. Oleson* (L 10850); Iowa City, *J. A. Perish* (Ia), July 29, 1923, *G. W. Martin* (Ia 1174); Spirit Lake, July 16, 1926, *G. W. Martin* (Ia 1077); Turkey Creek, Johnson Co., July 12, 1902, *B. Shimek* (Ia 1349).

Missouri: Creve Coeur, St. Louis Co., on wood, Aug. 7, 1891, *Dr. Lind* (?) (MBG 16363); Dixon, on *Betula*, Aug. 1930,

Marion Child (MBG), on *Platanus*, Aug. 1928, *Marion Child* (MBG); Meramec, on old wood, Nov. 1905, *P. Spaulding* (MBG); Pleasant Valley, St. Louis Co., on *Betula*, Sept. 1928, *Marion Child* (MBG); St. Louis, Forest Park, on *Ulmus*, Oct. 17, 1896, *H. von Schrenk* (MBG 43049), on dead trunks, July 4, 1898, July 19, 1902, *H. M. Glatfelter, 50* (MBG).

Nebraska: Weeping Water, April 19, 1924, *L. V. Walker* (W).

Texas: Houston, on fallen limbs, 1869, *H. W. Ravenel* (PDS).

EUROPE:

England: Kings Lynn, Norfolk, on decayed *Fraxinus excelsior*, 1874, *C. B. Plowright, 69* (F, PDS, Ia, St, L 10883), *C. B. Plowright, 216* (F); Crediton, on felled trees, Nov. 21, 1904, *Mrs. A. Montague* (L 10893); Dewesbury, on *Fraxinus*, *E. M. Wakefield* (PDS); Lancashire, Oct. 8, 1910, *H. J. Wheldon* (L 10872); London, *E. M. Holmes* (L 10828, 10895); *M. C. Cooke, 669* (F); Somerset, on dead trunks, June 1875, *Mac Owan & Tuck* (St 17); Southhampton, *M. C. Potter* (L 10894).

France: Pas-de-Calais, *C. Cepede* (L 10871, 10877); Seine-et-Oise, March–Aug. 1886, *F. Sarrazin* (MBG 16366).

Germany: Munich, on *Alnus glutinosa*, May 1910, *J. R. Weir, 2750* (PDS), on *Fagus*, 1910, *J. R. Weir, 2756* (PDS); Oberammergau, Aug. 1889, in Fungi Bavarici, *Schnabel, 76* (F, St 32); Reigenwalsermunde, on *Alnus incana*, 1892, *P. Sydow* (St 8); without locality (St 21).

Austria: Vorarlberg, on beech, winter, 1898, *J. Rick, 1228* (F, St); Wiener Wald, Nov. 3, 1890, *von Höhnel, 4350* (F).

Sweden: Bygget, *C. G. Lloyd* (L 10898); Upsala, 1909, *C. G. Lloyd* (L 10879).

Finland: Kuusamo, *Karsten, 265*, in Fungi Fennici (F).

Switzerland: 1821, *Fries, 141*, TYPE collection (F).

Italy: on *Alnus glutinosa*, 1923, *Bresadola* (PDS); Lombardy, 1889 (PDS); Trieste, on trunks of various dead trees, Sept. 1874, *Saccardo, 697* (PDS); without locality, *Cesati, 600* (F).

Rumania: Siebenbürgen, on *Betula alba*, Aug. 1883, *Linhart, 216*, in Fungi Hungarici (PDS, F).

Jugo-Slavia (Serbia): immature specimen (St).

Russia: Novogorad, Prov. of Bologoi, on *Acer platanoides*, July 6, 1918, Aug. 30, 1918, *W. Tranfeschel* (St 35, 129).

AFRICA:

Egypt, on *Betula alba*, Aug. 1883, *Römer, 261* (F).

ASIA:

Siberia: Prov. of Omsk, Tara Distr., on limbs of *Betula verrucosa*, Sept. 7, 1921, *Murashinsky* (PDS).

China: *Prof. H. H. Hu* (L 35857).

PACIFIC ISLANDS:

Japan: Iwanai, on rotten wood, July 12, 1916, *A. Yasuda, 7611* (PDS); Nayege, on dead *Pavonia cuspidata*, Aug. 1912, *Takisanjo Mikava* (L 10881); Sendai, May 1913, *A. Yasuda* (L 10880); Sakawa, Tosa, May 1919, *Prof. T. Yoshinaga* (L 10817).

Island of Timor: on trees, 1910, *M. Ferreina* (F).

AUSTRALIA: Victoria, on dead stump of dogwood, Oct. 1911, *Jas. Wilson* (L 10878).

2. Daldinia occidentale Child, sp. nov.

Pl. 27, fig. 6; pl. 28, fig. 3; pl. 32, fig. 4.

Stromata hemisphaeroidea vel subglobosa, sessilia, raro substipitata, carbonacea, plerumque solitaria, raro confluentia, 2.5–8 x 2–7 x 1–3.5 cm.; ectostromatibus tenuibus fragilibusque, primum "Hay's Brown" deinde "Deep Vinaceous Brown" demum obscure atris vel aereo-atris; entostromatibus fibroso-nemorosis, persistentibus, zonatis, zonis subaequalibus, zonis pallidioribus "Drab," "Tawny Olive Cinnamon," "Hair Brown" vel saepius "Benzo Brown," mollibus vel nemorosis, persistentibus, zonis obscurioribus "Bone Brown," "Fuscous" vel atris; peritheciis monostichis vel polystichis, irregulariter pyriformibus, 518–1554 x 185–518 μ; ostiolis punctiformibus vel minute papillatis, plerumque papillatis; sporidiis fuscis, ellipsoideis vel inaequilateraliter ellipsoideis, (9.6)–12.8–(19.2) x (4.8)–6.4–(8)–(9.6) μ.

Stromata hemispherical to subglobose, sessile or rarely substipitate, carbonaceous, usually single or occasionally confluent, 2.5–8 x 2–7 x 1–3.5 cm. Ectostroma at first "Hay's Brown," then "Deep Vinaceous Brown" and finally dull black or shiny

bronze-black, thin, extremely carbonaceous and brittle, cracking off and exposing the tips of the perithecia. Entostroma fibrous-woody, persistent, conspicuously zonate, the zones subequal, the darker ones "Bone Brown" or brownish-black, alternating with lighter zones of "Drab," "Tawny Olive Cinnamon," "Hair Brown," or usually "Benzo Brown" or "Fuscous," soft to woody, persistent. Perithecia monostichous, less frequently polystichous, irregularly pyriform, 518–1554 x 185–518 μ, the wall 14.8–44.4 μ thick; the ostioles punctiform to prominent, mostly prominent. Ascospores ellipsoid to inequilaterally ellipsoid, fuscous, (9.6)–12.8–(19.2) x (4.8)–6.4–8–(9.6) μ. Conidia ellipsoidal, hyaline, borne singly or in whorls, 4–6 x 2–3 μ.

On wood of deciduous trees. Central to western North America, Chile, Tasmania, and New Zealand.

This species, called *Daldinia* "Y" in a previous paper,[28] although closely related to *D. concentrica*, differs from it quite markedly when a series of specimens are studied. Not only are the fruiting bodies unlike, but the asexual conidia are also somewhat larger. In addition to such characters, there is the difference in the physiological behavior of the two species. It is therefore felt that the creation of this new species is fully warranted. See also fig. 4.

Lloyd[29] apparently recognized that the western American material that had been known as *D. concentrica* differed somewhat from the typical form and published it without description as *D. concentrica* forma *californica*. This material was from Washington State where *D. grande* is also found. Another specimen, from Idaho (12379), he labelled *D. confluens* because of its manner of growth. As far as the writer is aware, however, this species has not been published.

Specimens examined:

CANADA:

Ontario: London, on decaying wood, July 1904, *J. Dearness, 2013* (PDS, St), on *Pyrus sambucifolia*, Aug. 7, 1880, *C. G. Pringle, 1161* (F, St 27), *Rev. H. Dupret* (L 12312).

British Columbia: Bendigo, Victoria, June 1918, *E. J. Summers* (L 10806).

[28] Child, M. Ann. Mo. Bot. Gard. 16: 411–486. 1929.

[29] Lloyd, C. G. Myc. Writings 5: 23–26. *figs. 1452–1453.* 1919.

UNITED STATES:

Michigan: Vermilion, on dead birch stump, July 30, 1914. *A. H. W. Povah* (Po 229c), June 27, 1914, *A. H. W. Povah* (Po 617).

Wisconsin: Madison, Oct. 1901, *M. B. Nichols, 4* (St).

Minnesota: Cass Lake, on *Tilia americana*, June 1912, *J. R. Weir* (F); Minneapolis, *Mrs. M. S. Whetstone* (L 10911, 10912, 10930), *Daisy Hone* (L 10913, 10927); Monticello, *E. P. Ely* (L 10928, 10929).

North Dakota: on old log, Feb. 1897, *Prof. V. Reed* (F).

South Dakota: Black Hills, 1903, *von Schrenk*, TYPE (MBG 43121); Custer, on *Quercus* sp., Aug. 1, 1918, *J. R. Weir, 10857* (PDS).

Montana: Boulder, on *Populus trichocarpa*, Sept. 16, 1917, *F. S. Wolpert* (PDS, MBG 58395); Shelby, on standing *Alnus pyrifolia*, Oct. 13, 1911, *J. R. Weir, 6295* (PDS); Melrose, on *Salix* sp., Oct. 6, 1919, *E. E. Hubert* (PDS); Missoula, on *Acer* sp., 1913, *J. R. Weir, 6295* (PDS); Logan, on burned *Betula granulosa*, May 6, 1928, *P. A. Young, 76* (PDS).

Colorado: Eldorado, on dead *Populus tremuloides*, May 1913, *E. Bethel* (L 35856); Grand Mesa, Sept. 11–18, 1929, *F. J. Seaver & P. F. Shope* (NY 567).

Idaho: Boise, on limb of bearing *Pyrus Malus*, April 15, 1916, *E. P. Taylor, 1847* (PDS); Priest River, on fire-killed saplings of *Betula occidentalis*, Aug. 3, 1920, *A. S. Rhoads, 16434, 16440* (PDS), on *Betula papyrifera*, Aug. 28, 1920, *A. S. Rhoads, 16648* (PDS), on *Betula papyrifera*, Aug. 3, 1920, *A. S. Rhoads, 16450* (PDS), on *Alnus tenuifolia*, Aug. 10, 1920, *A. S. Rhoads, 16435* (PDS), on *Alnus tenuifolia*, Aug. 7, 1920, *J. R. Weir, 15055* (PDS), on *Alnus*, *J. R. Weir* (L 12379), on *Alnus tenuifolia*, June 1914, and Sept. 1914, *J. R. Weir, 2753, 2754, 7444* (PDS); Salmon, on *Populus trichocarpa*, July 15, 1917, *F. S. Wolpert, 7048* (PDS), on *Salix* sp., July 15, 1917, *F. S. Wolpert, 6851* (PDS); St. Maries, on *Populus trichocarpa*, Sept. 9, 1913, *J. R. Weir, 7817* (PDS).

Washington: Bellingham, on *Alnus rhombifolia*, Oct. 1913, *J. R. Weir, 2748* (PDS); Newport, on *Alnus tenuifolia*, Aug. 21, 1920, *A. S. Rhoads, 16613* (PDS); Metaline Falls, on fire-

killed standing *Betula papyrifera*, Aug. 27, 1920, *A. S. Rhoads, 16865* (PDS); Yakima, on prune branch, 1922, *Brooks* (S); Langley, 1922, *J. M. Grant* (PDS); Marysville, on *Alnus*, 1922, *J. M. Grant* (MBG 64435); Sequim, *J. M. Grant* (L 10936, 12378); Spokane, on *Betula papyrifera*, Aug. 8, 1924, *C. R. Stillinger* (L 11796).

California: Monterey, *T. H. MacBride* (Ia 1347), 1894, *T. H. MacBride* (Ia 1348); San Bernardino, May 30, 1899, *S. B. Parish* (L 10932), on fire-killed *Salix* sp., Feb. 13, 1920, *E. Bethel* (L 35855); Santa Paula, on *Citrus* tree, 1912, *H. S. Fawcett* (L 10925).

SOUTH AMERICA:

Chile: Santiago, on trunks, *M. R. Espinosa* (L 10920); Danquilco, Rio Puelo, on dead trunks, Jan. 1916, *M. R. Espinosa, 28* (PDS).

TASMANIA: Aug. 1889, *L. Rodway* (L 10843).

NEW ZEALAND: Caversham, *W. A. Scarfe* (L 10899); Christchurch, *Rev. J. Mitchell* (L 11759); Dunedin, *Helen K. Dalrymple* (L 10815); Otira, *E. Cheel* (L 10832); Waikanini, *W. E. Barker* (L 10844), *S. Duncan* (L 10896); Wellington, Island Bay, *Jessie Dunn* (L 10897); without locality, Oct. 1911, *W. E. Barker* (L 10900).

3. Daldinia grande Child, sp. nov.

Pl. 27, fig. 8; pl. 28, figs. 7–8; pl. 30, fig. 8; pl. 32, fig. 3.

Stromata sessilia, subhemisphaeroidea, late confluentia, carbonacea, nemorosa, 1.2–14 x 1.5–7.5 x 1–5 cm.; ectostromatibus primum "Dark Vinaceous Brown" deinde obscure atris vel aereo-atris, valde fragilibus; entostromatibus dense fibrosis, persistentibus, manifesto zonatis, zonis obscurioribus "Fuscous Brown" vel "Bone Brown," zonis pallidioribus "Drab," "Hair Brown" vel "Benzo Brown," mollibus vel dense fibrosis persistentibusque, 2–3-plo latioribus; peritheciis monostichis vel polystichis, praecipue polystichis, pyriformibus vel subclaviformibus, 518–1850 x 185–740 μ; ostiolis raro punctiformibus, saepe prominentibus, mammiformibus; sporidiis ovoideis vel inaequilateraliter ellipsoideis, dilute fuscis vel atro-fuscis, (8)–14.4–16–(27.2) x (6.4)–8–(11.2) μ.

Stromata sessile, flattened-hemispherical, usually broadly confluent, carbonaceous, woody, 1.2–14 x 1.5–7.5 x 1–5 cm. Ectostroma at first "Dark Vinaceous Brown," finally dull black to bronze-black, extremely brittle. Entostroma densely fibrous, persistent, conspicuously zonate, the darker zones "Fuscous" to "Bone Brown," the lighter zones 2–3 times broader, soft to fibrous, "Drab," "Hair Brown," or "Benzo Brown." Perithecia monostichous or polystichous, pyriform to subclaviform, 518–1850 x 185–740 μ, the walls 14.8–59.2 μ thick; ostioles rarely obsolete or punctiform, usually prominent and mammiform. Ascospores ovoid, ellipsoid to inequilaterally ellipsoid, the ends bluntly rounded, fuscous to deep fuscous, (8)–14.4–16–(27.2) x (6.4)–8–(11.2) μ.

On wood of various deciduous trees. Western United States, Ecuador, Australia, and New Zealand.

This species differs from the preceding in that the perithecia are polystichous as a rule rather than as an exception; the ostioles are more prominent as papillate or mammiform protuberances on the surface of the stroma; and the spores are somewhat larger and more bluntly rounded at the ends.

Specimens examined:

UNITED STATES:

Minnesota: Cass Lake, on roots of living *Tilia americana*, *J. R. Weir, 2752* (PDS).

Montana: Melrose, on *Salix* sp., Oct. 6, 1919, *E. E. Hubert* (PDS).

Colorado: on aspen, *Ernest Knäbel* (L 10826, 10944).

Arizona: Soldiers Camp, Coronado National Forest, on dead *Populus tremuloides*, *G. G. Hedgcock* (L 11769).

Washington: Bellingham, on *Acer macrophyllum*, 1914, *J. R. Weir, 2755* (PDS); Langley, 1922, *J. M. Grant* (PDS); without locality, 1912, 1916, *T. H. MacBride* (Ia).

California: Sacramento, on cottonwood stump, April 1909, *Miss Sutcliff* (L 10941); San Bernardino, on *Salix*, May 24, 1920, *E. Bethel* TYPE (Sh); San Francisco, Golden Gate Park, on *Acacia* sp., Oct. 16, 1910, *W. T. Swingle* (PDS); Victorville, on dead area of *Salix Gooddingii*, April 4, 1918, *E. Bethel* (L 11768); southern California, 1899, *S. B. Parish* (Ia 1346).

South America:
Ecuador: Quito, *Rev. L. Mille* (L 10829); *N. Nuñez* (L 10831).
Australia: East Caulfield, *J. T. Paul* (L 10838, 10919).
New Zealand: Christchurch, *J. Mitchell* (L 11795); Dunedin, *E. P. Northcroft* (L 12296); Tapanui, Otago, *R. G. Robinson* (L 10889) Waikawa, *W. E. Barker* (L 10901).

4. Daldinia Eschscholzii (Ehrenb.) Rehm, Ann. Myc. **2**: 175. 1904.

Sphaeria Eschscholzii Ehrenberg, Fungi Cham. 89. *pl. 18, fig. 8.* 1820.
Daldinia concentrica var. *Eschscholzii* (Ehrenb.) Rehm, Ann. Myc. **2**: 175. 1904.
Daldinia vernicosa f. *microspora* Starbäck, Kongl. Svensk Vet-Akad. Handl. III. **27**[9]: 6. 1901, sense of Theissen.
Daldinia concentrica var. *microspora* (Starb.) Theissen, Ann. Myc. **7**: 3. 1909.
Daldinia corrugata Patouillard & Hariot, Bull. Soc. Myc. France **22**: 120. 1906.
Daldinia argentinensis f. *sessilis* Spegazzini, of authors in part, Anal. Mus. Nac. Buenos Aires III. **12**: 345. 1909.
Daldinia luzonensis Rehm, Philippine Jour. Sci. **8**: 260. 1913.
? *Hypoxylon stratosum* Sacc. Syll. Fung. **9**: 544. 1891.
? *Daldinia stratosa* Sacc. Syll. Fung. **22**: 327. 1913.

Pl. 26, fig. 5; pl. 27, fig. 5; pl. 28, fig. 5; pl. 29, figs. 3, 5–6, 8; pl. 31, fig. 1; pl. 33, fig. 3.

Stromata subhemispherical, flattened, solitary or confluent, sessile or substipitate, usually smooth, rarely deeply wrinkled or convolute, 1.5–7 x 1.7–5.5 x .8–4 cm. Ectostroma thin, brittle, usually "Anthracene Purple," "Dark Vinaceous Brown," or "Sorghum Brown," later becoming black, dull or laccate. Entostroma soft to pithy, but persistent, conspicuously concentrically zonate, the lighter zones "Pale Mouse Gray," "Mouse Gray," "Light Drab," "Pale Drab," "Smoke Gray," "Tilleul Buff," "Hair Brown," or "Benzo Brown," radiate-fibrous and two or

more times broader than the darker "Bone Brown" or "Fuscous" zones. Perithecia usually monostichous, very rarely polystichous, closely appressed, claviform, 518–1628 x 148–518 μ, the walls 14.8–51.8 μ thick. Ostioles distant, usually obsolete to punctiform, rarely prominent. Ascospores amber-colored to brown, inequilaterally ellipsoid, the ends usually lighter-colored and refractive, (8)–11.2–(14.4) x 4.8–(6.4) μ.

On the wood of various deciduous trees. Tropical or subtropical.

This species is closely related to *Daldinia concentrica*, in that it has a dense, dark-colored entostroma, but aside from this point there is little in common, for the spores are smaller and the perithecia, entostroma, and other characters show evident differences in appearance. However, during the examination of a large number of specimens, it was found that when the frequency of spore sizes was plotted the median fell at 11.2 x 4.8 μ, but within this grouping there were nevertheless four rather distinct classes of spore sizes of the following frequency: 39 collections of which the median size was 11.2 x 4.8 μ; 17 collections at 11.2 x 6.4 μ; 16 collections at 12.8 x 6.4 μ; 9 collections at 12.8 x 4.8 μ. Although there is such a variation in spore size, it is not singular that a species with such a wide distribution should show some variation.

The type of *D. corrugata* from Africa was not available for examination, but several specimens so classified in the Lloyd herbarium proved to be small specimens of *D. Eschscholzii*. Infolding of the stroma, alone, does not seem to be a character of sufficient taxonomic value to warrant its use in the creation of a new species. In the original description, Patouillard and Hariot describe the entostroma as white and zonate. None of the specimens seen by the writer has a truly white entostroma, but rather a light gray one. This point may be one that depends on the personal equation, and hence should not be too strongly stressed. According to Lloyd,[30] this African species is quite close to *D. concentrica* in spite of its corrugated surface. The writer feels that this species should be considered a synonym of *D. Eschscholzii*. Similarly, *D. luzonensis*, of which the type and co-type

[30] Lloyd, C. G. Mycological Writings 5: 25. *fig. 1454.* 1919.

material were examined, proved to be synonymous with this species.

Specimens examined:

EXSICCATI: Klotzsch, Herb. Viv. Myc., 162; Maire, Mycoth. Bor.-Afr., 120; Rehm, Ascomyceten, 1718; Roumeguére, Fungi Select. Exsicc., 5140, 5829; Smith, C. L. Cent. Amer. Fungi, 33; Wright, Fungi Cub. Wright., 818.

UNITED STATES:

Florida: MacLendons I. (probably Florida), on *Citrus decumana* stump, *W. T. Swingle, 1697* (F, MBG); Gainesville, *N. L. T. Nelson* (L 10923, 10942, 12179), June 4, 1930, and Aug. 1930, *E. West* (MBG); Victoria, Orange Co., on felled maple, July 20, 1919, *C. H. Baker, 557* (L); on grubbed orange stump, Dec. 20, 1923, *A. S. Rhoads* (L 11771); on *Citrus Bigaradia*, April 25, 1892, *W. T. Swingle* (PDS).

Louisiana: St. Martinsville, on rotten hardwood limb, June 24, 1911, *C. J. Humphrey* (L 10809); Pointe à la Hache, on decaying log, July 1886, *Rev. A. B. Langlois, 825* (F), Aug. 1896, *Langlois, 829* (PDS).

Texas: Feb. 23, 1921, *N. L. T. Nelson, 1* (L 10814, St); Austin, Dec. 1930, *G. W. Goldsmith* (T).

MEXICO: Vera Cruz, *C. L. Smith, 1351* (Ia).

CENTRAL AMERICA:

Nicaragua: Los Amates, Feb. 20, 1907, *W. A. Keller* (PDS); Ometepé, Jan.–Feb. 1893, *C. L. Smith, 33* (F); Castillo Viejo, Feb.–Mar. 1893, *C. L. Smith, 78* (PDS).

Honduras: La Ceiba, May 1923, *C. M. Sutter* (MBG 18576); Tela, on dead tree trunks, Jan. 17, 1923, *O. A. Reinking* (L 12294).

WEST INDIES:

Cuba: Ciego, on dead roots, Dec. 5, 1924, *J. R. Weir, 66519* (PDS), on dead roots in cane field, Dec. 5, 1924, *J. R. Weir, 6519* (F); Consolacion, on fallen trunk, Dec. 1, *C. Wright, 818* (F); Guantanamo Oriente, July 1920, and Dec. 28, 1920, *B. Hioram, 16579, 17769* (PDS); Palo Seco, on *Citrus*, Dec. 13, 1915, *J. A. Stevenson, 2757* (PDS); Santa Clara, Nov. 23, 1924, *J. R. Weir* (S); Santiago de las Vegas, Dec. 10, 1915, *J. R. Johnston, 1335* (S); Trinidad, Nov. 24, 1924, *J. R.*

Weir, 64816 (PDS); without locality, *C. G. Lloyd* (L 10836, 10839, 10847).

Porto Rico: Pueblo Viejo, on rotten stumps, Jan. 1915, *J. A. Stevenson, 2525* (PDS); Rio Piedras, on logs, Dec. 4, 1915, *B. Fink, 657* (PDS); San Juan, on dead *Citrus* stump, March 1, 1917, *H. E. Thomas, 8* (S); without locality, Feb. 30, 1919, *F. S. Earle, 247* (S), Dec. 6, 1920, *F. S. Earle, 309* (PDS).

Jamaica: *Wm. Chadwick* (L 10868).

Bahamas: *L. J. K. Brace* (L 10813, 10830, 12302, 12406); *A. D. Machado* (L 12385).

St. Croix: Jolly Hill, 1905–06, *C. Raunkiaer, 1711a* (B).

SOUTH AMERICA:

Argentina: La Plata, on *Quercus ruber*, Dec. 1923, *J. R. Weir* (PDS); Rio Pescado, Jujuy, on decayed trees, March 1905, *Spegazzini* (A), (1).[31]

Bolivia: Tatarenda, Gran Chaco, March 4, 1902, *Robt. E. Fries, 408* (St).

Brazil: *J. Rick* (L 12303); Leno Agul (?), 1923, *J. Rick* (L 10851); Bahia, June 1915, *C. Torrend, 15811* (PDS); Matto Grosso, Cuyabá, May 13, 1894, *G. O. Malme, 0595* (St).

EUROPE:

France: Dijon, Côte d'Or, 1904, *M. Barbier* (L 10841).

Germany: Nassau, in forest of Hattersheim, *Fuckel, 305* (F, PDS, St).

AFRICA:

Algiers: on trunk of dead *Eucalyptus*, Feb. 3, 1913, *R. Maire, 120* (PDS, St); on trunks, Oct. 1889, *L. Trabut, 5140* (F); Coomassee, *T. Hunter* (L 10846).

Liberia: on burnt stump, July 25, 1926, *D. H. Linder, 167* (F); Mt. Barclay, on charred stumps and logs, July 20, 1926, *D. H. Linder* (F); *T. Hunter* (L 10823).

Gold Coast: Abeesé, *R. H. Bunting* (L 10834).

Nigeria: 1917, *Farquharson, 5* (PDS).

São Thomé I.: on fallen trunks, *O. Möller, 5829* (F).

Congo: (L 10867); *F. Theissen* (L 10938).

[31] Since the type collection was a mixed collection, having no number, the author has separated the material and put it into packets bearing the numbers 1, 2, and 3. See footnote on page 477.

Angola: Loanda, on dead trees, March 1918, *J. Gossweiler* (L 10849); *J. Gossweiler* (L 12376).

Union of South Africa: Stellenbosch, *Miss A. V. Duthie* (L 10904); Pretoria, *A. J. T. Janse* (L 10875).

Mozambique: Tiembo, April 1913, *A. Truz, 142* (St).

Tanganyika Territory (Dutch East): Tanga, *A. Karansek* (L 10862).

Uganda Protectorate: on logs, Feb. 19, *R. A. Dammer, 1442* (PDS).

Zanzibar: *Chas. A. O'Connor* (L 12180).

MADAGASCAR: *Henri Perrier de la Bathie* (L 10856).

REUNION ISLAND: *E. Dupont* (L 10890).

MAURITIUS ISLAND: *Chas. A. O'Connor* (L 10891).

ASIA:

Syria: Beyrouth, *Rev. A. Boulomoy* (L 10858).

India: Pusa, on old wood, May 14, 1906, *Inayat* (St 1121), on wood, Feb. 1923, *Azmabullah Khan* (PDS), on *Dalbergia Sissoo*, Feb. 1, 1917, *E. J. Butler* (PDS), on same host, Aug. 1910, *J. F. Dastur* (St 1122); Bombay, on old wood, Oct. 3, 1908, *H. M. Chibber* (St 1118); Madras, Oct. 1911, *J. Hornell* (L 10845); Nagpur, (on living ?) *Citrus Aurantium*, Sept. 19, 1908, *Paudit* (St 1115); Burma, on old wood, Jan. 12, 1908, *E. W. Buttel* (St 1120).

Malay Peninsula: Negri Sembilan, on dead *Hevea*, Nov. 22, 1922, *R. E. Holttum* (L 12806); Singapore, on dead log, Dec. 29, 1919, *T. F. Chipp* (L 10821), on dead rubber stump, Nov. 1923, *Abul Vadir* (L 12297).

Bonin Island: 1853, *C. Wright, 56* (F).

China: Kuling, Prov. of Kiangsi, Sept. 8, 1929, *Chung, 4355* (F).

PHILIPPINE ISLANDS: Capez, Panay, Jan. 10, 1904, *E. B. Copeland* (L 10802); Palawan, July 1912, *E. Fenix, 15613* (L 12407, PDS); Leyte, Palo, Jan. 1906, *A. D. E. Elmer* (MBG 705734); March 1909, *A. Celestina, 21* (St); Prov. of Bataan, Luzon, Nov. 1912, *P. W. Graff* (L 10803); Antipalo, Luzon, Oct. 1912, *M. Ramos* (St 16824, as *D. concentrica* var. *microspora*, authentic material, L 10801); Mt. Maquiling, Luzon, on rotten trunks, Jan. 31, 1914, *C. F. Baker, 2723* (St), on dead

wood, Oct. 4, 1920, *S. Bacal* (L 10173, PDS), on dead wood, Oct. 3, 1920, *R. Habaluijas* (L 10598), Nov. 1920, *S. Mariano* (L 10686, PDS), on dead wood, Oct. 1920, *O. A. Reinking* (L 10613, PDS), Oct. 3, 1920, *C. Serrano 9934* (L 12182), Oct. 3, 1920, *A. Reyes* (L 10800), on *Garcinia* sp., Nov. 7, 1920, *A. Reyes* (L 10950, PDS), on dead wood, Oct. 1920, *G. Zabella* (L 10919, PDS), Dec. 1919, *H. S. Yates* (L 10587), July 5, 1919, *J. Corrales* (L 10797); Prov. of Nueva Vizcaya, Luzon, Jan. 1913, *McGregor* (PDS 20274); Prov. of Tayabas, Luzon, 1916, *H. S. Yates* (MBG 59960); Manila, *Father Sanchez* (L 11878, St), on *Hevea brasiliensis*, Oct. 1917, *H. S. Yates 96* (PDS); Isla Cruz, Prov. of Laguna, Luzon, on *Citrus decumana*, Feb. 2, 1919, *N. Reyes* (L 10805); Prov. of Calauan, Laguna, on dead wood, Feb. 13, 1921, *F. Bernardo* (L 12292), *O. A. Reinking* (L 12291); Prov. of Rizal, Luzon, Oct. 12, *M. Ramos* (St); Butuan, Subprov. Mindanao, March–July, 1911, *C. M. Weber, 1257* (F); Los Baños, Prov. of Laguna, Luzon, on dead wood, Nov. 7, 1920, *A. Abesans* (L 10852), on rotten trunks, Dec. 1, 1912, **TYPE** collection of *D. luzonensis* Rehm, *C. F. Baker* (MBG 12465, L 12401), Oct. 2, 1919, *H. Cuzner* (L 10798), on decaying wood, Sept. 25, 1919, *G. O. Ocfemia* (PDS 5944), on bark of *Tamarindus Medicus*, Aug. 5, 1913, *Evaristo, 1562, 1568* (St), on dead wood, March 1919, *E. Quisunging* (PDS 3598), on decaying wood, Sept. 25, 1919, *G. O. Ocfemia* (PDS, Po 229b, L 10799); Benquet, Los Baños, on *Polyalthia, O. A. Reinking, 11370* (PDS), on *Celtis*, April 2, 1921, *O. A. Reinking, 11262* (PDS), on dead wood, April 2, 1921, *O. A. Reinking* (L 12290, 12293), on *Dipterocarpus*, April 2, 1921, *O. A. Reinking, 66606* (PDS), on *Macaranga*, April 2, 1921, *O. A. Reinking, 11350* (PDS), on *Citrus decumana*, March 2, 1919, *N. Reyes, 3575* (PDS); Basilan I., Isabela, Dec. 1919, *H. S. Yates* (L 10587).

EAST INDIES:

Java: Cheribon, on *Tamarindus indicus*, 1920, *J. C. van der Meer Mohr* (S), 1922, ex *H. Bigoriensis* (MBG); Pati, on *Dalbergia latifolia* (dead), March 1920, *C. Hartley, 19861* (PDS); Buitenzorg, 1913, *W. P. Thompson* (F), *C. Hartley,*

19661 (PDS); Madiun, Jan. 29, 1922, *Dr. T. A. Tengwall* (L 12299).

Timor Island: *G. Bresadola, 45* (PDS), on trunks, 1916, *M. Feniera, 42* (PDS).

Australia: Sydney, *E. Cheel* (L 10827), *W. W. Froggatt* (L 10902), *J. B. Cleland* (L 10876); Grantville, *F. F. Paul* (L 10892); without locality, Dec. 1919, *H. S. Yates* (L 10585, 12409).

Tasmania: *L. Rodway* (L 10816).

Pacific Islands:

Samoa: 1904–05, *C. G. Lloyd* (L 10859, 10860, 10869, 12400, 12298); Apiaberg, on *Citrus Aurantium*, May 1905, *Dr. K. & E. Rechinger, 1718* (B, F, PDS, St), authentic material; 1904–05, *W. A. Setchell & H. E. Parks* (L 12298).

Guam: *Peter Nelson* (L 12387).

Tahiti: Tataa, on dead cocoanut, May, 1922, *H. E. Parks* (L 12394), on rotting *Papaya*, June 1922, *H. E. Parks* (L 12295).

5. Daldinia Bakerii Lloyd, Myc. Writings **5**: 25–26. 1919, char. emend.

Daldinia Eschscholzii (Ehrenb.) Rehm, of authors.

Pl. 31, fig. 5.

Stromata subhemisphaeroidea vel subsphaeroidea, plerumque convoluta, solitaria vel confluentia, sessilia vel substipitata, levia vel rugosa plicataque, 1.5–4.5 x 2–4 x 1.2–2.3 cm.; extostromatibus carbonaceis subfragilibus, "Deep Vinaceous Brown" vel "Deep Livid Brown" deinde fulgidis vernicosisque, atris; entostromatibus mollibus, contextis vel fibrosis, persistentibus, manifesto zonatis, zonis obscurioribus "Benzo Brown," "Bone Brown" vel "Fuscous," zonis pallidioribus "Pallid Mouse Gray," "Dark Mouse Gray," "Smoke Gray" vel "Hair Brown," 2–3 plo latioribus; peritheciis plerumque monostichis, raro polystichis, plerumque late claviformibus, 666–8510 x 148–592 μ; ostiolis prominentibus, dense aggregatis, saepe ad bases stromatum majoribus; sporidiis inaequilateraliter ellipsoideis, intus ascos obliquiter monostichis, dilute fuscis vel atro-fuscis, (9.6)–14.4–(24.0) x (4.8)–6.4–(11.2) μ.

Stromata subhemispherical to globose, usually convolute, single or confluent, sessile to substipitate, smooth or wrinkled and infolded, 1.5–4.5 x 2–4 x 1.2–2.3 cm. Ectostroma carbonaceous, somewhat fragile or brittle, "Deep Vinaceous Brown" to "Deep Livid Brown," later becoming black, shining, and laccate. Entostroma soft, pithy to fibrous, persistent, zonate, the lighter zones "Pallid Mouse Gray," "Dark Mouse Gray," "Smoke Gray" to "Benzo Brown," 2–3 times wider than the darker "Benzo Brown," "Bone Brown," or "Fuscous" zones. Perithecia usually monostichous, more rarely polystichous, mostly broadly claviform, widest at apices, but slightly tapering at the base, 666–8510 x 148–592 μ, the wall 22.2–51.8 μ thick; ostioles prominent, close together, frequently more prominent at the base of the stroma. Spores inequilaterally ellipsoid, obliquely uniseriate in the ascus, amber-colored to dark brown, with or without refractive ends, (9.6)–14.4–(24.0) x (4.8)–6.4–(11.2) μ.

On wood of various deciduous trees. Asia, Africa, Philippine Islands, Australia, Costa Rica, Porto Rico, Mexico, West Indies, and South America.

This species is closely related to *D. Eschscholzii*, but because of the wider range in the size of the spores and also their generally larger size, it is considered to be distinct.

Specimens examined:

MEXICO: Mexico City, on *Fraxinus* (*Berlandieriana* ?), June 25, 1924, *Dr. A. Dampf, 6350* (PDS); without locality, *W. A. Murrill, 0313* (L).

CENTRAL AMERICA:

Costa Rica: Estrella Valley, on decayed prostrate log, July 1, 1921, *Paul V. Siggers* (L 12178).

WEST INDIES:

Porto Rico: on old stump, Dec. 7, 1920, *F. S. Earle, 301* (S).

SOUTH AMERICA:

Argentina: Chaco, Prov. of Jujuy, Oct. 6, 1901, *R. E. Fries* (St 053).

Bolivia: Tarija, Jan. 21, 1902, *R. E. Fries* (St 246).

Chile: Chaparro, Limache, Sept. 23, 1927, *Garaventa* (F).

AFRICA:

Natal: Pretoria, Aug. 1918, *J. M. Sim* (L 10824); without locality, *A. J. T. Janse* (L 10870).

ASIA:

Asia Minor: Turkey, Palu, Aug. 1923, *Remp* (PDS).

India: Saharanpur, *Wm. Golden* (L 10857); Cuttack, *Jages Ray* (L 10837); Bengal, *S. Hutchings* (L 10855, 10842); Dadabetta, Oct. 1911 (L 10866); Darjeeling, *G. H. Cave* (L 10863); Pusa, on old wood, April 8, 1906, *Inayat* (St 1124); Cherra Punji, on old wood, May 15, 1905, *E. W. Butler* (St 1119); Nilgiri, on old wood, Aug. 1902, *C. A. Barber* (St 1117).

PHILIPPINE ISLANDS: Luzon, Prov. of Tayabas, Dec. 1916, *H. S. Yates, 25604* (F); Subprov. Panai, Jan. 1915, *M. S. Clemens, 9246* (F); Subprov. Benquet, Jan. 1915, *M. S. Clemens* (MBG 59959); Batangas, on dead branch of *Citrus nobilis*, Oct. 17, 1917 (S); Los Baños, Prov. of Laguna, on dead wood, May 6, 1917, *F. Collado* (PDS 3632).

AUSTRALIA: Sydney, 1901, *R. T. Baker*, TYPE (L 12377).

6. **Daldinia Gollani** Hennings, Hedwigia **40**: 339. 1901.

Daldinia cognata Patouillard & Hariot, Jour. de Bot. **17**: 15. 1903.

Hypoxylon Hibisci Hennings, Hedwigia **47**: 259. 1908.

Daldinia platensis Spegazzini, Anal. Mus. Nac. Buenos Aires III. **12**: 345. 1909.

Daldinia Hibiscus (Henn.) Lloyd, Myc. Writings **6**: 901. *fig. 1587*. 1919.

Pl. 27, fig. 7; pl. 31, fig. 6.

Stromata flattened-hemispherical, turbinate to subglobose, sessile to substipitate, 1–3.5 x 1–4 x .8–1.7 cm. Ectostroma usually rugose, thin, brittle, "Hay's Brown," "Brownish Drab," "Dark Vinaceous Brown," "Anthracene Purple," later dull or laccate and black. Entostroma soft, pithy or fibrous, persistent, concentrically zonate, the lighter zones "Tilleul Buff," "Hair Brown," "Gray White," "Drab Gray," or "Benzo Brown," 1–6 times wider than the darker "Bone Brown" or "Fuscous" zones. Perithecia usually monostichous, rarely polystichous, globose, 444–1250 x 162–518 μ, the walls 22.2–51.8 μ thick; ostioles distant, obsolete, punctiform or slightly papillate. Ascospores inequilaterally ellipsoid, acutely rounded at the lighter colored ends, amber to brown, (6.4)–9.6–(12.8) x (3.2)–4.8–(6.4) μ.

On wood of deciduous trees. Jamaica, Brazil, Argentina, New Caledonia, India, and the Philippine Islands.

This species differs from all others in the *D. concentrica* group by having subglobose perithecia, and conspicuously acutely rounded spores of relatively small size.

Specimens examined:

WEST INDIES:

Jamaica: Balaclava, May 4, 1909, *A. E. Wight, 340* (F).

SOUTH AMERICA:

Brazil: Rio Grande do Sul, Lageado, 1925, *Rick* (PDS 19811); without locality, *Rick* (PDS, L 12383, 12389); Matto Grosso, Sierra da Chapada, June 20, 1894, *G. O. Malme, 5* (St).

Argentina: La Plata, on trunks, Dec. 28, 1906, *Spegazzini*, TYPE of *D. platensis* (A).

ASIA:

India: Siwalik Range, 1600 ft., on *Ficus Carica*, 1900, *W. Gollan, 269*, TYPE (B).

PHILIPPINE ISLANDS: without data (L 12408), probably TYPE of *D. Hibiscus; E. D. Merrill* (L 12403).

NEW CALEDONIA: 1901, *Desmazieres*, TYPE of *D. cognata* (L 12374).

7. Daldinia caldariorum Hennings, Verh. Bot. Ver. Prov. Brandenb. **20**: 158. *pl. 2, fig. 14.* 1897.

Pl. 26, figs. 6–7; pl. 31, fig. 2.

Stromata subglobose, laterally compressed, 5–12 x 5–12 x 5 mm. Ectostroma thin, brittle, at first "Hay's Brown" or "Dark Vinaceous Brown," finally shining black. Entostroma definitely zonate, the lighter zones "Hair Brown," soft, compact, persistent, twice as broad as the "Fuscous" zones. Perithecia monostichous, irregularly pyriform, 666–814 x 296–444 μ; the walls 29.6–37 μ thick. Ostioles obsolete. Ascospores brown, inequilaterally ellipsoid to ellipsoid, (9.6)–11.2–(14.4) x 4.8–(6.4) μ.

On wood of deciduous trees. Germany and Brazil.

Specimen examined:

GERMANY: Berlin Botanical Garden fern house, Dec. 1887, *P. Hennings* TYPE (B). Hennings[32] also reports the occurrence of this species from Brazil.

[32] Hennings, P. Broteria **5**: 50. 1906.

8. **Daldinia loculata** (Lév.) Sacc. Syll. Fung. **1**: 394. 1882.
Sphaeria loculata Léveillé, Ann. Sci. Nat. Bot. III. **3**: 47. 1845.
Daldinia intermedia Lloyd, Myc. Writings **5**: 23–26. 1919.
Daldinia Murrillii Lloyd, Myc. Writings **6**: 901. *fig. 1588.* 1919.

Pl. 26, figs. 3–4; pl. 30, fig. 4; pl. 32, figs. 1–2.

Stromata globose above, usually rugose, .3–2 x .5–2.3 x .6–2.5 cm., sharply constricted below the perithecia-bearing head to a definite cylindrical stipe, rarely substipitate, usually single, rarely confluent. Ectostroma thin, moderately brittle, at first "Hay's Brown," "Brownish Drab" or "Deep Vinaceous Brown," later becoming black, dull or laccate. Entostroma conspicuously zonate, densely fibrous, persistent, the lighter zones "Benzo Brown," "Hair Brown," or "Fuscous," "Mouse Gray" or "Smoke Color," 2–5 times wider than the darker "Bone Brown" zones. Perithecia monostichous or polystichous, pyriform to irregularly subclaviform, subdistant, 185–518 x 444–1480 μ; ostioles obsolete, punctiform, to rather prominent, distant to subdistant. Ascospores navicular to inequilaterally ellipsoid, amber-color to brown, the ends often lighter in color and refractive, (9.6)–12.8–(19.2) x (4.8)–6.4–(8) μ.

On wood. United States, Mexico, West Indies, Japan.

Of the species with the dense, persistent entostroma, only this one has a definite stipe, and by this character it can be readily recognized.

Specimens examined:

UNITED STATES:

New York: Menaud, 1845, *J. H. Léveillé*, TYPE (P).

South Carolina: Claw Hammer Cove, Pisgah, on *Hicoria alba*, Aug. 15, 1924, *G. G. Hedgcock* (L 11767); on dead limbs of *Ostrya virginica*, *H. W. Ravenel* (F).

Ohio: Preston, 1896, *A. P. Morgan* (Ia); Akron, *G. D. Smith* (L 12404).

Michigan: East Lansing, *E. A. Bessey* (L 12329).

Wisconsin: Cleveland, *Chas. Golosel* (L 12405).

Minnesota: Princeton, on dead *Carpinus caroliniana*, Sept. 26,

1911, *C. J. Humphrey* (L 12305); Minneapolis, *Mary S. Whetstone* (L 12307).

Iowa: Delaware Co., *B. Shimek* (Ia 1352).

MEXICO: *W. A. Murrill*, TYPE of *D. Murrillii* (L 12402).

WEST INDIES: Jamaica, 1908 and 1909, *A. E. Wight* (F).

ASIA:

Japan: Kobe, *J. E. A. Lewis* (L 11793); Sendai, Mt. Akagi, Oct. 9, 1911, *A. Yasuda* (L 12167).

9. Daldinia vernicosa (Schw.) Cesati & de Notaris, Comm. della Soc. Critt. Ital. **1**: 198. 1863.

Sphaeria concentrica var. *stipitata* Pers. Syn. Meth. Fung. **1**: 8. *pl. 1. figs 3–4.* Göttingen, 1801.

Hypoxylon concentricum var. *obovatum* Fries, Syst. Myc. **2**: 331. 1823.

Sphaeria vernicosa Schw. Jour. Acad. Nat. Sci. Phila. **5**: 9. *pl. 1. fig. 2.* 1825.

Sphaeria (*concentrica*) *cingulata* Lév. Ann. Sci. Nat. Bot. III. **3**: 47. 1845.

Hypoxylon (*Pulvinatae*) *vernicosum* (Fr.) Berk. & Curt. Proc. Linn. Soc. Bot. **10**: 384. 1867.

?*Daldinia cingulata* (Lév.) Sacc. Syll. Fung. **1**: 395. 1882.

Daldinia fissa Lloyd, Myc. Writings **7**: 1313. *pl. 306, fig. 2986.* 1924.

Pl. 26, fig. 1; pl. 28, fig. 1; pl. 30, figs. 2, 5; pl. 33, fig. 2.

Stromata subturbinate to turbinate, contracted below into a distinct rugose stipe with annular zones externally visible, stromata usually single, occasionally confluent, fragile, .6–3.5 x .5–2.5 x .8–2.5 cm. Ectostroma thin and carbonaceous, brittle, at first "Hay's Brown, "Cameo Brown," "Van Dyke Brown," "Fuscous" or "Dark Vinaceous Brown," finally shiny or laccate and black. Entostroma conspicuously zonate, the lighter zones usually gray-white, yellowish gray, or "Drab," fibrous, collapsing and loculate, 2–4 times broader than the darker and more persistent "Bone Brown" to black zones. Perithecia monostichous or polystichous, ovoid-oblong to subglobose, 518–1258 x 185–

666 μ, the wall 14.8–37 μ thick. Ostioles punctiform to somewhat prominent. Ascospores usually brown or dark brown, the ends obtusely rounded, (8)–11.2–12.8–(17.4) x (4.8)–6.4–(8) μ. Conidia single or in whorls, subglobose to ovoid, hyaline, 2.4–3.2 x 2.4–3.2 μ.

On wood, frequently charred, of living and dead deciduous trees. Cosmopolitan.

The substipitate, turbinate stroma, together with the light colored entostroma, marks this species.

Daldinia cingulata and *D. fissa* appear to be ill-advised species which were founded on large or abnormal specimens. The writer could find no characters other than that of size to separate the latter species from *D. vernicosa*. From the early descriptions and illustrations, *H. concentricum* var. *obovatum* is clearly synonymous with *D. vernicosa*, and as such it has been pretty generally accepted, although the writer has found some specimens of *D. Eschscholzii* which have been determined as this variety. *Sphaeria concentrica* var. *stipitata* is apparently synonymous with *D. vernicosa*. Plate figures show definite stipitate ascocarps and a marked differentiation of color in the zonation, which was evidently intended to show light gray or white loculate zones alternating with more dense dark ones. The ascocarps of *D. concentrica* are sessile or substipitate and the zones are shades of brown and are more dense than those of *D. vernicosa*.

Specimens examined:

Exsiccati: de Thümen, Fungi Austr., 1154; Ell. & Ev., Fungi Columb., 735; Ellis, N. Am. Fungi, 166; Fairman, Mycoth. Fairmani, 4415; Fuckel, Fungi Rhenani, Suppl., 2468; Linhart, Fungi Hung., 180; Roumeguére, Fungi Gall. Exsicc., 3946; Sacc. Mycoth. Veneta, 153; Sydow, Mycoth. Marchica, 3469.

United States:

New Hampshire: Meredith, Sept. 1924, *Miss Ann Hibbard* (L 11876).

Vermont: Middlebury, Oct. 1910, *C. G. Lloyd* (L 12325).

Massachusetts: Cambridge, on *Fagus*, Sept. 1915, *A. P. D. Piguet, 10* (F); Canton, on *Tupelo*, Oct. 19, 1929, *D. H. Linder* (Li).

Connecticut: Sand Beach, *Miss Ruby Wilbur* (L 12315).

New York: Lyndonville, on old birch poles, autumn, 1920 or 1921, *C. E. Fairman* (S 4415); Syracuse, Aug. 1911, and Sept. 11, 1913, *L. H. Pennington* (L 12319 and 12321).

New Jersey: Newfield, on dead white oak saplings, Sept. 1899, *Ellis* (F), Ellis, N. Am. Fungi, on dead shrubs and trees, *Ellis* (PDS 1175).

Pennsylvania: State College, on dead *Quercus alba*, Aug. 31, 1916, *A. S. Rhoads* (L 11770); Philadelphia, Aug. 31, 1904, *Mrs. G. M. Dallas* (L 12330).

Maryland: Glen Echo Heights, on *Liriodendron tulipifera*, April 19, 1925, *G. G. Hedgcock* (L 11875); Hyattsville, on *Quercus*, Nov. 1, 1885, *F. L. Scribner* (PDS), Nov. 1, 1887, *F. L. Scribner, 441* (PDS); Holland Point, on *Ilex opaca*, March 25, 1923, *A. Marlowe & E. K. Cash* (PDS), on *Cornus florida*, *E. K. Cash* (PDS); Scotland, St. Marys Co., on dead *Ilex opaca*, Sept. 2, 1923, *S. F. Blake* (PDS); Cabin John, on fire-killed *Acer rubrum*, Oct. 21, 1917, *A. S. Rhoads* (L 12386).

District of Columbia: Washington, on dead wood, March 26, 1916, *W. Piper* (PDS), on *Fagus atropunicea*, Aug. 30, 1914, *R. G. Pierce* (PDS 8240); *Dr. A. Hrdlicka* (L 12323).

Virginia: Arlington, on *Quercus*, *Hicoria*, *Liriodendron*, and *Sassafras*, Feb. 22, 1927, *C. L. Shear* (S 5586), on wild grape vine, Dec. 11, 1911, *J. R. Weir, 2760*, (PDS); Clarendon, on fire-scarred *Liriodendron*, Aug. 28, 1926, *J. R. Weir* (PDS), on *Diospyros virginiana*, Oct. 10, 1926, *J. R. Weir* (PDS 66661).

North Carolina: Salem, on posts of garden fence, 1825, *Schweinitz*, TYPE (Pa).

South Carolina: North Augusta, *Berry Benson* (L 12313).

Georgia: Tallulah Falls, Sept. 19, 1901, *A. B. Seymour* (F).

Florida: De Funiak Springs, *G. C. Fisher* (L 12324).

Louisiana (?): on *Quercus macrocarpa*, *A. B. Langlois* (PDS).

Ohio: Akron, *G. D. Smith* (L 12310); Salem, on ash, *B. Leeper* (L 12311); Toledo, *W. R. Lowater* (L 35854), and TYPE of *D. fissa* (L 12382).

West Virginia: Pendleton Co., on *Fagus ferruginea*, Sept. 17,

1904, *A. H. Moore 2321* (F); Fairmont, *Rev. A. Boutlou* (L 12328); Radnor, on burned *Quercus alba*, Nov. 28, 1920, *C. L. Shear* (S).

Michigan: Croton, on dead white oak, Sept. 20, 1914, *C. D. LaRue* (Po 617A, L 12318); Mt. Pleasant, Dec. 1913, *A. E. Jenkins* (PDS).

Indiana: Sandusky, on dead *Quercus alba*, Aug. 17, 1912, *C. J. Humphrey* (L 12317); Scottsburg, on dead *Quercus alba*, *J. R. Weir 21070* (PDS).

Wisconsin: Green Bay, on dry *Fagus*, July 24, 1889, *J. W. Schuette* (PDS); Shawano, on healthy plum limbs, June 14, 1897, *W. S. Wood* (PDS).

Illinois: Evanston, Oct. 4, 1909, *L. H. Pennington* (L 12320).

Minnesota: Minneapolis, *Mary S. Whetstone* (L 12306, 12308); Rochester, *J. E. Greene* (L 12309).

Iowa (?): *T. H. MacBride* (Ia 1419).

Missouri: St. Louis Co., on dead trunk, April 6, 1919, *S. M. Zeller* (MBG 12326); Old Orchard, Oct. 1886, *Pammel* (MBG 66605); *T. H. MacBride* (Ia 1383); Dixon, on fire-killed *Acer rubrum*, July 1928, *Marion Child* (MBG); Lesterville, on charred *Quercus*, Nov. 1926, *L. O. Overholts* (MBG 63847).

North Dakota: Kulm, on *Fraxinus*, Dec. 14, 1913, *J. F. Brenckle, 2758* (PDS, St 678).

Kansas: Fort Riley, on oak poles, Oct. 1917, *J. F. Brenckle, 99* (F, L, PDS).

Texas: College Station, *L. W.* (L 10812).

Montana: Darby, on *Alnus tenuifolia*, July 1915, *J. R. Weir, 6298* (PDS).

Idaho: Priest River, on *Alnus tenuifolia*, Oct. 1915, *J. R. Weir, 2761* (PDS).

Washington: Chelan Lake, on *Alnus tenuifolia*, Sept. 1916, *J. R. Weir, 2759* (PDS).

Oregon: Corvallis, *S. M. Zeller* (MBG).

MEXICO: (Ia 1353); Jalapa, *C. L. Smith* (Ia 1415).

CENTRAL AMERICA:

Honduras: *O. A. Reinking* (L 12316).

WEST INDIES:

Cuba: Oriente, Guantanamo, on lianas, 1918, *B. Hioram, 12486* (PDS).

South America:

Brazil: Minas Geraes, 1877, *F. Noack* (St 267).

Europe:

France: Seine-et-Oise, *F. Sarrazin, 3946* (F).

Italy: Treviso, on dead trees, Sept. 1894 (F), on trees, Sept. 1872, *P. A. Saccardo* (St 18); L. Maggiore, autumn of 1865 (St 361); Vercelli, *Cesati* (St 600).

Germany: Berlin, on trees stumps, 1890–91, *Sydow, 3469* (St, F).

Austria: Eberbach, on *Fagus* trunks, *Fuckel, 2468*, in Fungi Rhenan. (F); on *Alnus*, Oct. 26, 1904, *von Höhnel, 4350* (F).

Hungary: Wieselburg, on *Betula alba*, Nov. 1882, *Linhart, 180* (F).

Rumania: Transylvania, Dec. 1872, *C. Barth, 1154* (F, PDS, St).

Russia: Majkop (?), on fallen logs, *N. Schestunow* (L 10882).

Philippine Islands: Manila, on fallen trunks, 1914, *A. Morgan, 12641* (PDS).

10. Daldinia simulans Child, sp. nov.

Pl. 26, fig. 2; pl. 28, fig. 2; pl. 30, fig. 3; pl. 33, fig. 4.

Stromata elongato-ellipsoidea vel subglobosa, substipitata vel breve-stipitata, plerumque solitaria, raro confluentia, sursum minute sulcata, "Snuff Brown," "Drab," vel "Verona Brown," 5–15 x 14–15 x 9–13 mm; stipitibus sterilibus zonatis, atris, fulgidis; ectostromatibus tenuibus; entostromatibus fibrosis, partim collabascentibus loculatisque, zonatis, zonis atris tenuibus, zonis pallidioribus "Pale Dull Gray," 4–5-plo latioribus; perithecis monostichis, pyriformibus vel late ovoideis, 444–888 x 185–518 μ; sporidiis fuscis vel atro-fuscis, ellipsoideis vel subinaequilateraliter ellipsoideis, (9.6)–11.2–(14.4) x (4.8)–6.4–(8.) μ; conidiis solitariis vel verticillatis, hyalinis, elongato-ellipsoideis, 8–12.8 x 4.4 μ.

Stromata elongate-ellipsoidal or subglobose, substipitate or short-stipitate, usually single, occasionally confluent, minutely

transversely sulcate above, "Snuff Brown," "Drab," or "Verona Brown," 5–15 x 14–15 x 9–13 mm.; sterile stipe zonate, black and shining. Ectostroma thin. Entostroma fibrous and partially collapsing, somewhat loculate, zonate, the thinner zones thin and black, the lighter zones 4–5 times broader, "Pale Dull Gray." Perithecia monostichous, subdistant, pyriform to broadly ovoid, 444–888 x 185–518 μ, the walls 29.6–37 μ thick. Ascospores fuscous to deep fuscous, ellipsoid to subinequilaterally ellipsoid, (9.6)–11.2–(14.4) x (4.8)–6.4–(8) μ. Conidia solitary or in whorls, hyaline, elongate-ellipsoid, 8–12.8 x 4.4 μ.

On wood of deciduous trees. Ohio, Illinois, and Missouri.

This species is close to *D. vernicosa* but differs from it by the usually smaller ascocarps, which are brownish rather than purplish and in young material are somewhat transversely rugose. The stipe, also, is more conspicuously annulate from the internal concentric zones. Physiologically this species, previously known as *Daldinia X*, is also distinct, as has been reported by the writer.[23]

Specimens examined:

UNITED STATES:

Ohio: Cincinnati, Sept. 22, 1920, *C. G. Lloyd* (L 11758).

Illinois: Evanston, Sept. 29, 1928, *H. Fox* (Po 229e).

Missouri: Valley Park, Sept. 1929, *D. H. Linder*, TYPE (Li, MBG); Dixon, Aug. 1929, *Marion Child* (MBG).

11. Daldinia albozonata Lloyd, Myc. Writings 5: 822. *fig. 1374.* March, 1919; *ibid.* 25. *fig. 1456.* July, 1919 (char. emend.).

Pl. 27, fig. 3; pl. 31, fig. 3.

Stromata mutua pressione irregulariter turbinata, dense caespitosa, subito in stipites crassos attenuata, .5–2 x 1–2 x .5–3 cm.; ectostromatibus "Dark Vinaceous Drab," "Dusky Drab," deinde "Anthracene Purple" vel atris, numquam fulgidis; stipitibus manifesto zonatis, longitudinaliterque rugosis, nemorosis; entostromatibus contextis vel fibrosis, persistentibus, non collabascentibus, manifesto zonatis, zonis obscurioribus "Bone Brown," fibrosis, zonis pallidioribus "Tilleul White," 4–5 plo latioribus; peritheciis late claviformibus, ad bases attenuatis, non dense

[23] Child, M. Ann. Mo. Bot. Gard. 16: 411–486. 1929.

aggregatis, in totis peripheris stromatum, 148–370 x 370–740 μ; ostiolis prominentibus; ascis non persistentibus; sporidiis dilute fuscis, cymbiformibus, (6.4)–8–(9.6) x (2.4)–3.2–(4.8) μ.

Stromata irregularly turbinate by mutual pressure, densely cespitose, substipitate, .5–2 x 1–2 x .5–3 cm. Ectostroma "Dark Vinaceous Drab" to "Dusky Drab," finally "Anthracene Purple" to dull black or bronze-black, never shining; the stipe conspicuously zonate and longitudinally rugose, woody. Entostroma pithy, fibrous, persistent, conspicuously zonate, the lighter zones "Tilleul White" and 4–5 times broader than the darker "Bone Brown" zones. Perithecia borne over the entire stroma, broadly claviform, long-tapering at the base, subdistant, 148–370 x 370–740 μ, the walls 37 μ thick; ostioles prominent. Ascospores navicular, light fuscous, (6.4)–8–(9.6) x (2.4)–3.2–(4.8) μ.

The material upon which Lloyd based the species was sent from the Cameroons in Africa. As he remarks, it does suggest *D. vernicosa* because of the white inner context, which is firm, and a dull rather than a shining ectostroma. According to Lloyd, the ascospores measure 4 x 8 μ, but the writer has found them to be somewhat more variable and, for the most part, narrower.

Specimens examined:

AFRICA:

French Equatorial Africa (Cameroons): *G. Zenker*, TYPE (L 12375).

Angola: *J. W. Gossweiler* (L 12395).

Uganda: Mevu (?), alt. 1900 m. (PDS); Magungo (?), alt. 1300 m., *Dr. O. Mattiook*, *Balbo* (PDS).

12. Daldinia cuprea Starbäck, Kongl. Svensk Vet.-Akad. Handl. III. **27**[9]: 5. *fig. 2.* 1901.

Daldinia granulosa Spegazzini, Anal. Mus. Nac. Buenos Aires III. **12**: 345. 1909.

Pl. 27, fig. 1; pl. 28, fig. 6; pl. 30, fig. 7; pl. 31, fig. 4.

Stromata narrowly clavate, solitary, the stipe differentiated from the fertile head, conspicuously annularly zoned and rugose, 1.2–1.7 x 1–1.5 x 5–6.6 cm. Ectostroma thin, at first "Seal Brown," "Sorghum Brown," becoming "Dark Vinaceous Brown," and

finally black and laccate. Entostroma conspicuously zonate, the lighter zones "Tilleul Buff," "Light Buff," "Cinnamon" to "Pinkish Buff," or "Mouse Gray," fibrous to pithy, collapsing and loculate, 3–4 times broader than the darker and more persistent "Bone Brown" zones. Perithecia confined to the head, monostichous, napiform, 740–888 x 444–518 μ; the wall 22.2–29.6 μ thick. Ostioles large, prominent, close together. Ascospores navicular, amber-colored, the ends acutely rounded, lighter-colored, and refractive, (8)–9.6–(11.2) x 4.8 μ.

On wood of deciduous trees. Paraguay and Argentina.

This species is characterized by the presence of papillate ostioles and by the fact that the stipe, conspicuously annulate exteriorly, is sharply separated from the perithecium-bearing upper portion of the stroma.

A comparison of the types of *D. cuprea* and *D. granulosa* shows conclusively that the two species are identical.

Specimens examined:

SOUTH AMERICA:

Paraguay: Paraguari, Cerro Negro, Aug. 8, 1893, *G. O. Malme*, TYPE (St 6).

Argentina: Rio Pescado, Jujuy, March 1905, *Spegazzini*, TYPE of *D. granulosa* (A).

Island of Ta (probably in Argentina): April 1, 1902, *R. E. Fries* (St 373), and April 10, 1902, *R. E. Fries* (St 425).

LOCALITY UNKNOWN: March 22, 1902, *R. E. Fries* (St 337).

13. Daldinia clavata Hennings, Hedwigia **41**: 14. 1902.

Daldinia vernicosa var. *microspora* Starbäck, Kongl. Svensk Vet.-Akad. Handl. III. **27**[9]: 6. 1901.

Daldinia barbata Rick, Broteria **5**: 50. 1906.

Daldinia argentinensis Spegazzini, Anal. Mus. Nac. Buenos Aires III. **1**: 68. 1902.

Daldinia argentinensis Speg. f. *sessilis* of authors in part, Anal. Mus. Nac. Buenos Aires III. **12**: 345. 1909.

Pl. 27, fig. 2; pl. 30, fig. 6; pl. 33, fig. 5.

Stromata cylindrical to broadly clavate, sessile or contracted below into a short somewhat rugose stipe, single, rarely confluent,

laterally compressed, smooth or finely rugose, 1–6 x .9–4.5 x 1–5 cm. Ectostroma thin, brittle, at first "Hay's Brown" or "Dark Vinaceous Brown," finally dull to shiny black and laccate. Entostroma persistent, definitely zonate, the lighter zones "Tilleul Buff," "Hair Brown," "Drab Gray," or "Vinaceous Buff," fibrous to pithy and 4–5 times broader than the darker and more compact "Bone Brown" zones. Perithecia monostichous or polystichous, claviform, borne over almost the entire stroma, 740–1086 x 236–370 μ; the walls 22.2–29.6 μ thick. Ostioles distant, obsolete to punctiform. Ascospores amber-colored, navicular to subinequilaterally ellipsoid, ends acutely rounded, lighter colored, and refractive, (8)–9.6–(12.8) x (3.2)–4.8–(6.4) μ.

On wood of deciduous trees. Mexico, Guatemala, Brazil, and Argentina.

The obsolete ostioles and the conspicuously collapsing tissue of the entostroma separate this species from *D. cuprea*. It is further differentiated by the fact that the fertile portion of the fruiting body is not clearly distinguishable from the short stipe.

Specimens examined:

MEXICO: Vera Cruz, on trees, Dec. 1907, *C. A. Purpus* (L 12390); Mexico City, *S. Bonansea* (L 12391).

CENTRAL AMERICA:

Guatemala: *T. J. Collins* (L 12380); *Silas L. Schumo* (L 12397).

SOUTH AMERICA:

Brazil: Matto Grosso, Guia, May 13, 1894, *G. O. Malme*, authentic material of *D. vernicosa* var. *microspora* (St); Rio Grande do Sul, Lagedo, *Rick* (L 12304, 12393), 1921, *Rick* (PDS 19805); Rio Grande do Sul, 1904, *Theissen* (F) probably authentic; St. Catharina Island, Blumenau, 1892, *A. Möller*, TYPE (B).

Argentina: Missiones, Puerto Pampa, Jan. 1901, *E. Kermes*, TYPE of *D. argentinensis* (A); Jujuy, Rio Pescado, on dead trees, March 1905, *Spegazzini*, TYPE of *D. argentinensis* f. *sessilis* (2) (A).[34]

[34] The number in the bracket was used by the author in order to separate Spegazzini's mixed collection, which bore no number. The original packet contained a mixed collection and the author has separated the specimens accordingly: No. 1, *D. Eschscholzii;* No. 2, *D. clavata;* No. 3, *Hypoxylon* sp.

Doubtful or Excluded Species

Daldinia angolensis (Welw. & Curr.) Sacc. Syll. Fung. **1**: 394. 1882.

Hypoxylon angolense Welw. & Curr. Trans. Linn. Soc. Bot. **26**: 282. 1868.

The writer has been unable to examine the type material of this species, although she has studied specimens in the Lloyd Herbarium that pass under this name. In none of these, zonation is evident, hence the species should probably be placed in *Hypoxylon* in accordance with Welwitsch and Currey.[35] However, since the species has been placed in *Rhopalopsis*, *Kretzschmaria*, and *Camillea*, the writer feels that it is inadvisable to make a definite disposition of the species.

Daldinia aspera Massee, Kew Bull. Misc. Inf. **1898**: 134. 1898.

There is no evidence of definite zonation in the type material of this species. It probably should be considered a large tropical species of *Hypoxylon*, either close to or synonymous with *H. cerebrinum* Fée.

Daldinia asphalatum (Link) Sacc. Syll. Fung. **1**: 394. 1882.

Sphaeria asphalatum Link et Fries, Linnaea **5**: 540. 1830.

The entostroma of the type of this species is homogeneous. The writer has found this species under *D. Eschscholzii*, *D. occidentale*, and *D. Gollani*. It is probably a species of *Hypoxylon*.

Daldinia durissima (Schw.) Sacc. Syll. Fung. **1**: 394. 1882.

Sphaeria durissima Schw. Syn. Fung. Car. Suppl. 32. 1822.

This species is doubtfully *D. concentrica*. A photograph of the type was available for study (pl. 30, fig. 1), as was also a preparation of the ascospores. From a photograph it was impossible to see any evidence of zonation in the entostroma. The spores, however, are of the same size as those of *D. concentrica*. According to Cooke,[36] this is a synonym of *Hypoxylon marginatum*.

[35] Welwitsch, F. & F. Currey. Trans. Linn. Soc. Bot. **26**: 282. 1868.
[36] Cooke, M. C. Grevillea **11**: 121–140. 1883.

Daldinia exsurgens (Mont.) Rehm, Ann. Myc. **7**: 4. 1909.

Hypoxylon exsurgens Mont.-Guy. Syll. Gen. Spec. Crypt. 213. 1856.

This species was placed in *Daldinia* because of the very faint zones present in the entostroma. The type material, although showing the faint zones, appears to be referable to *Hypoxylon* near *H. herculeum*.

Daldinia Feei Sacc. Syll. Fung. **1**: 395. 1882.

This species was originally described by Fée[37] in *Sphaeria*, but from that genus it was transferred to *Daldinia* by Saccardo,[38] who, however, renamed it, since the name *D. vernicosa* was preëmpted. Later, Saccardo[39] with good reason transferred the species to *Xylaria* where it properly belongs. *D. Feei* is therefore synonymous with *Xylaria vernicosa* (Fée) Saccardo.

Daldinia placentiformis (B. & C.) Theissen, Ann. Myc. **7**: 4–5. 1909.

Hypoxylon (*Glebosae*) *placentiforme* Berk. & Curt. Fungi Cubensis, 383. 1867.

The homogeneous context of the authentic material seen by the writer excludes this species from *Daldinia*.

Daldinia stratosa Sacc. Syll. Fung. **22**: 327. 1913.

Hypoxylon stratosum Sacc. Syll. Fung. **9**: 544. 1891. Ita nuncupandum a *D. Eschscholzii* (Ehrenbg.) Rehm, Sacc. Syll. Fung. **17**: 617. 1905.

The writer has seen no specimen of this species. Saccardo has called it a *D. Eschscholzii*.

Daldinia Thouarsiana (Lév.) Sacc. Syll. Fung. **1**: 395. 1882.

Sphaeria Thouarsiana Lév. Ann. Sci. Nat. Bot. III. **5**: 258. 1846.

The context of the type material is homogeneous. The species should be placed in *Hypoxylon*. It has been variously considered as either *H. Thouarsianum* or *H. malleolus*.

[37] Fée, A. L. A. Nouv. Mém. Soc. Sci. Agr. et Arts du Dept. Bas Rhin **2**[1]: 143–146. *pl. 12, fig. 2.* 1834.

[38] Saccardo, P. A. Syll. Fung. **1**: 395. 1882.

[39] *Ibid.* **9**: 530. 1891.

Daldinia Warburgii Hennings, Fungi Warburg. Hedwigia 32: 225. 1893.

The type material shows a homogeneous context. The ascospores are a very dark brown color and are extremely long and narrow. The material examined obviously belongs in *Hypoxylon*.

Explanation of Plate

PLATE 26

All photomicrographs were taken at a magnification of approximately × 815.

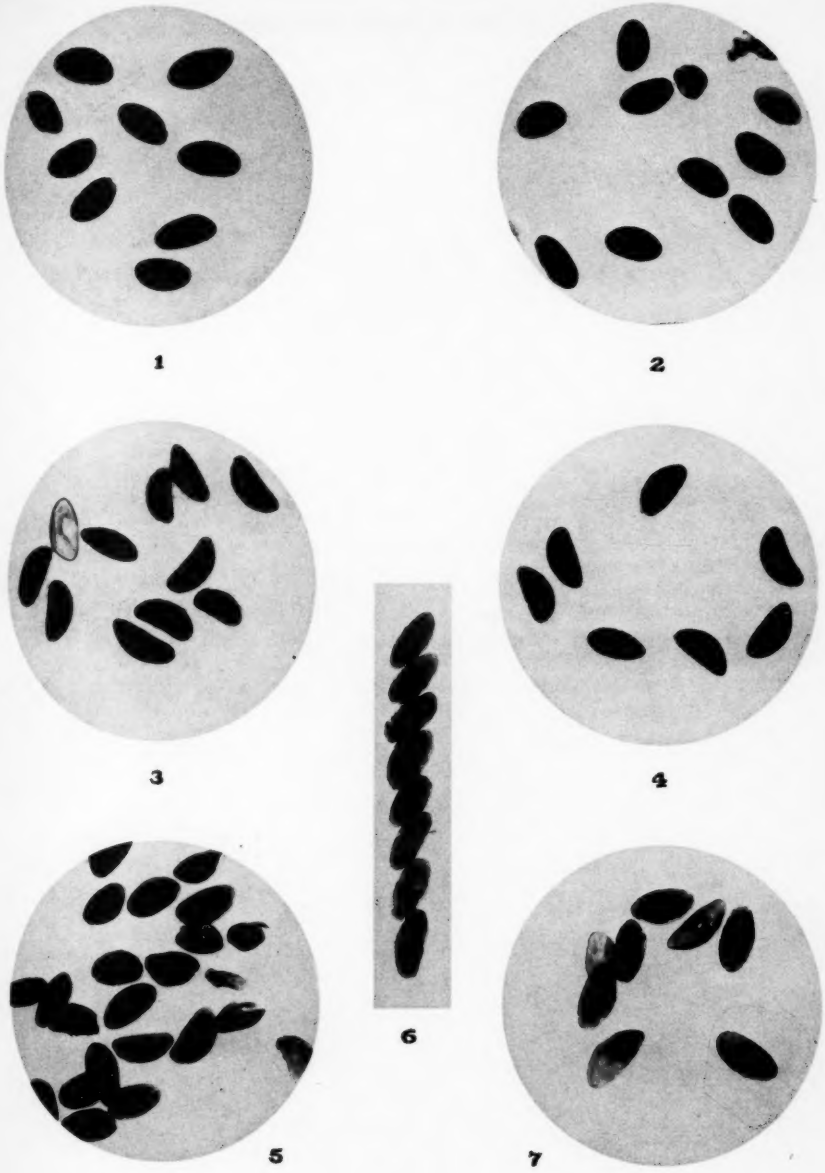
Fig. 1. *Daldinia vernicosa*, from material collected in Missouri (Li).

Fig. 2. *Daldinia simulans*, from type material.

Figs. 3–4. *Daldinia loculata*.

Fig. 5. *Daldinia Eschscholzii*, from material collected in the Bahamas, by A. D. Machado (L 12385).

Figs. 6–7. *Daldinia caldariorum*, from type material.



CHILD—THE GENUS DALDINIA

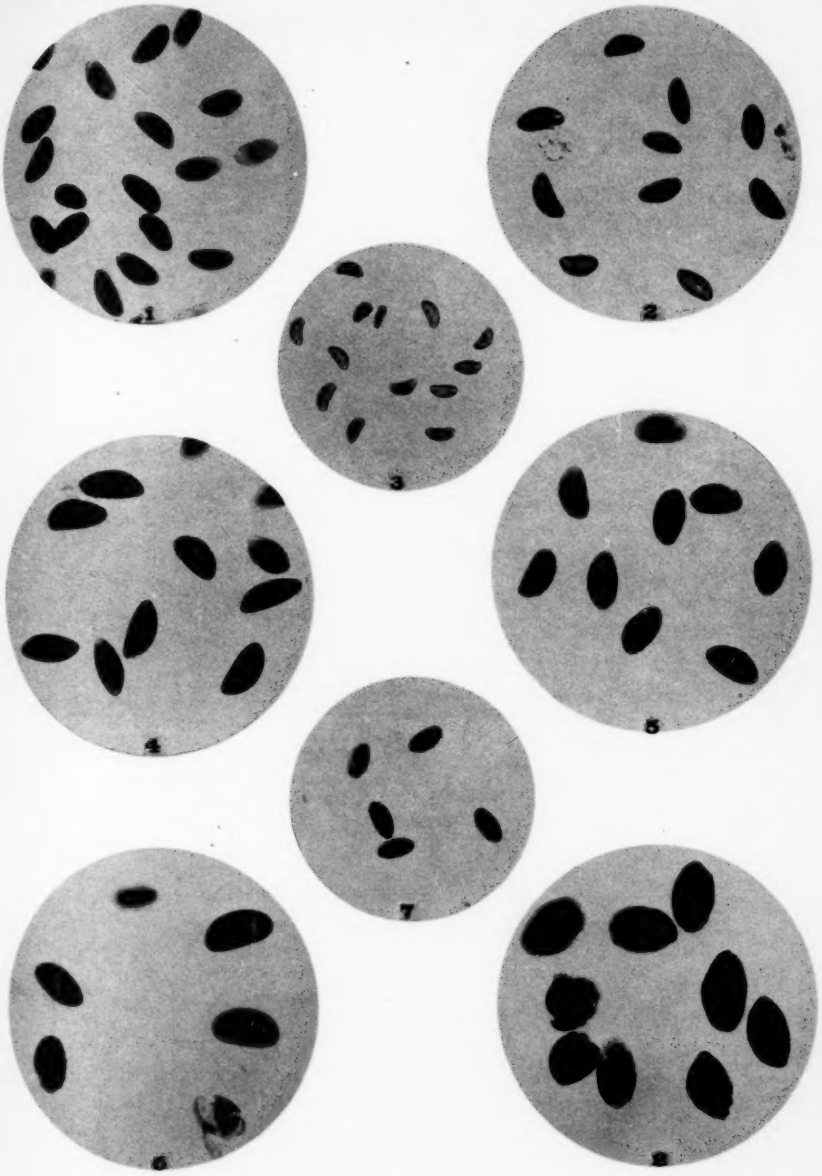
COCKAYNE, BOSTON

Explanation of Plate

PLATE 27

All photomicrographs were taken at a magnification of approximately × 815.

Fig. 1. *Daldinia cuprea*, from type material.
Fig. 2. *Daldinia clavata*.
Fig. 3. *Daldinia albozonata*, from type material.
Fig. 4. *Daldinia concentrica*, from material collected in Missouri by P. Spaulding.
Fig. 5. *Daldinia Eschscholzii*.
Fig. 6. *Daldinia occidentale*, from type material.
Fig. 7. *Daldinia Gollani*, from type material.
Fig. 8. *Daldinia grande*, from type material.



CHILD—THE GENUS DALDINIA

COCKAYNE, BOSTON

Explanation of Plate

PLATE 28

The photomicrographs were all taken at a magnification of × 13.

Fig. 1. *Daldinia vernicosa.* Note the shining surface and the well-spaced ostioles.

Fig. 2. *Daldinia simulans.* In this species, especially in material that is not quite mature, the surface is characteristically wrinkled, and the ostioles are inconspicuous.

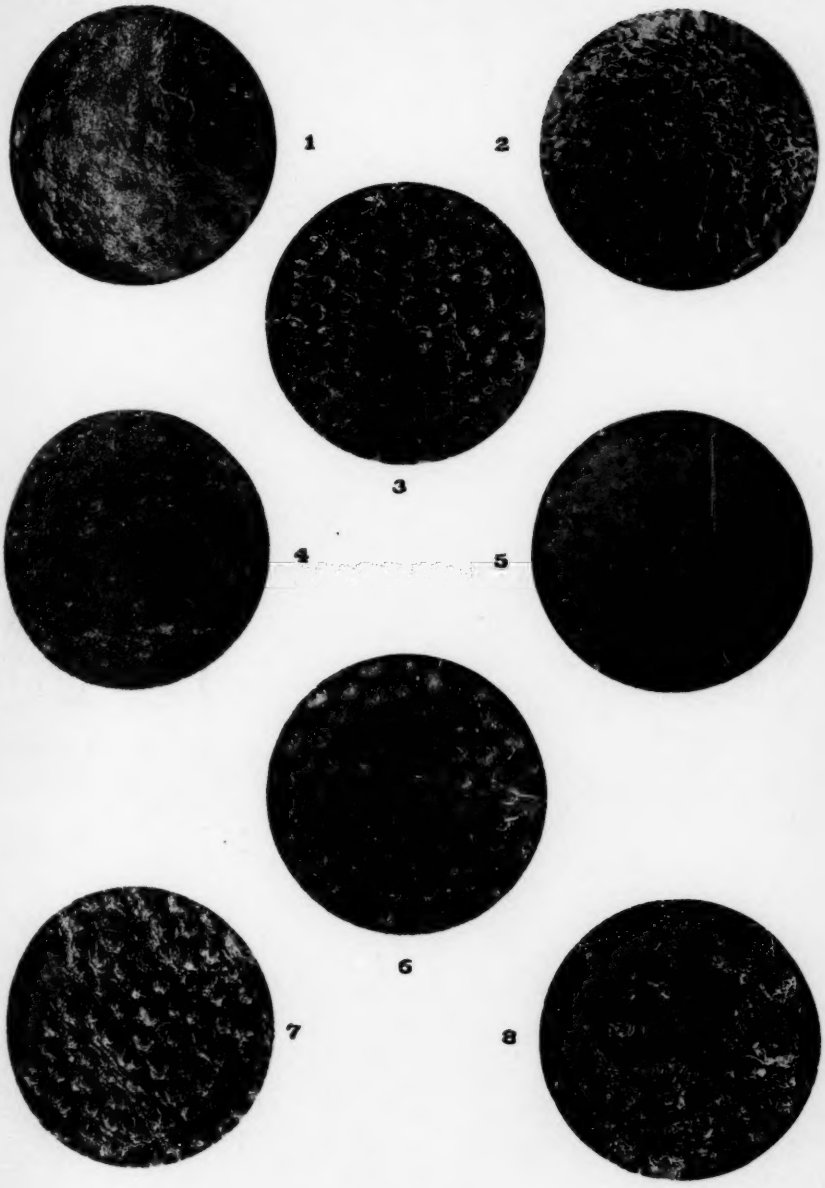
Fig. 3. *Daldinia occidentale.* The ectostroma is papillate around the ostioles, but the mouths of the ostioles are not pronounced.

Fig. 4. *Daldinia concentrica.* In this specimen the ostioles are not pronounced.

Fig. 5. *Daldinia Eschscholzii.* The ostioles in material that has not been rubbed or weathered are conspicuous as black dots.

Fig. 6. *Daldinia cuprea.* The ostioles are prominent and extend beyond the papillate ectostroma.

Figs. 7–8. *Daldinia grande.* The *Rosellinia*-like ostioles shown in fig. 8 are from the same fruiting body as are the smaller ones shown in fig. 7.



CHILD—THE GENUS DALDINIA

COCKAYNE, BOSTON

Explanation of Plate

PLATE 29

The scale accompanying each figure equals 1 centimeter.

Fig. 1. *Daldinia concentrica*, from Jugo-Slavia (St).

Fig. 2. *Daldinia concentrica*, from Germany (St). Note the rather prominent ostioles.

Fig. 3. *Daldinia Eschscholzii*, from Burma (St 1120).

Fig. 4. *Daldinia concentrica*.

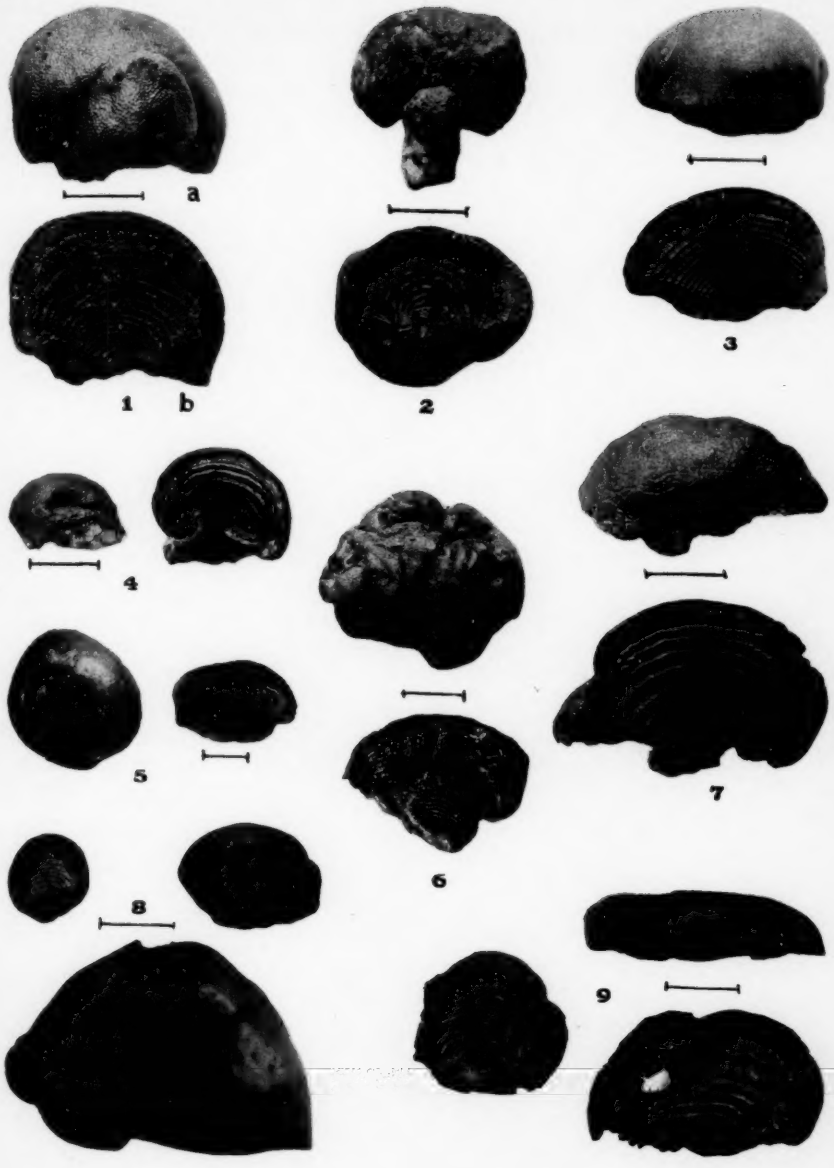
Fig. 5. *Daldinia Eschscholzii*, from the Philippine Islands, collected by M. Ramos at Antipalo, Luzon. Distributed from the Herbarium of the Bureau of Science at Manila, as *Daldinia concentrica* var. *microspora* (Starb.) Theiss.

Fig. 6. *Daldinia Eschscholzii* (Forestry Bureau no. 19201 of the Bureau of Science at Manila). This species with the convoluted stroma was distributed as *Daldinia concentrica*.

Fig. 7. *Daldinia concentrica* (Norfolk, England, *C. B. Plowright, 69* (St)).

Fig. 8. *Daldinia Eschscholzii*, from Mozambique.

Fig. 9. *Daldinia concentrica*, from Germany.



CHILD—THE GENUS DALDINIA

COCKAYNE, BOSTON

Explanation of Plate

PLATE 30

The scale accompanying each figure equals 1 centimeter.

Fig. 1. *Daldinia concentrica.* The type of *D. durissima* (Schw.) Sacc. The ostioles of the somewhat weathered material are rather prominent for this species.

Fig. 2. *Daldinia vernicosa,* from Canton, Massachusetts.

Fig. 3. *Daldinia simulans,* from Evanston, Illinois.

Fig. 4. *Daldinia loculata,* from Preston, Ohio. Note the definite, cylindrical stipe.

Fig. 5. *Daldinia vernicosa.* Type specimen in the Schweinitz Herbarium.

Fig. 6. *Daldinia clavata,* from Jujuy, Argentina, the type of *D. argentinensis* Speg.

Fig. 7. *Daldinia cuprea,* from Paraguay. Type material. The prominent ostioles and the less conspicuously zonate and loculate entostroma aid in separating this from the preceding species.

Fig. 8. *Daldinia grande.* Type material. Note the variation in the size and prominence of the ostioles.

CHILD—THE GENUS DALDINIA

Explanation of Plate

PLATE 31

The drawings of the perithecia were all made with the aid of the camera lucida and are all drawn to the same magnification.

Fig. 1. *Daldinia Eschscholzii*, drawn from a thick section and hence does not show the thickness of the ectostroma.

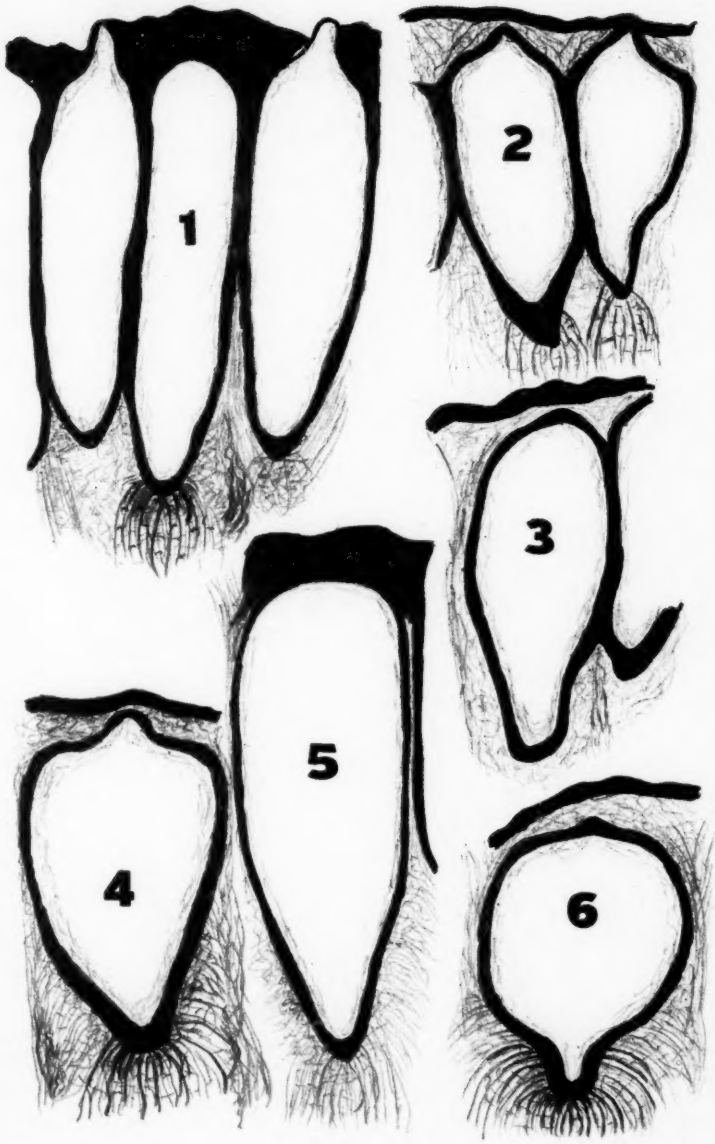
Fig. 2. *Daldinia caldariorum*, from type material.

Fig. 3. *Daldinia albozonata*, from type material.

Fig. 4. *Daldinia cuprea*, from specimen collected in Argentina (?) (St).

Fig. 5. *Daldinia Bakerii*, from type material.

Fig. 6. *Daldinia Gollani*, from type material.



CHILD—THE GENUS DALDINIA

Explanation of Plate

PLATE 32

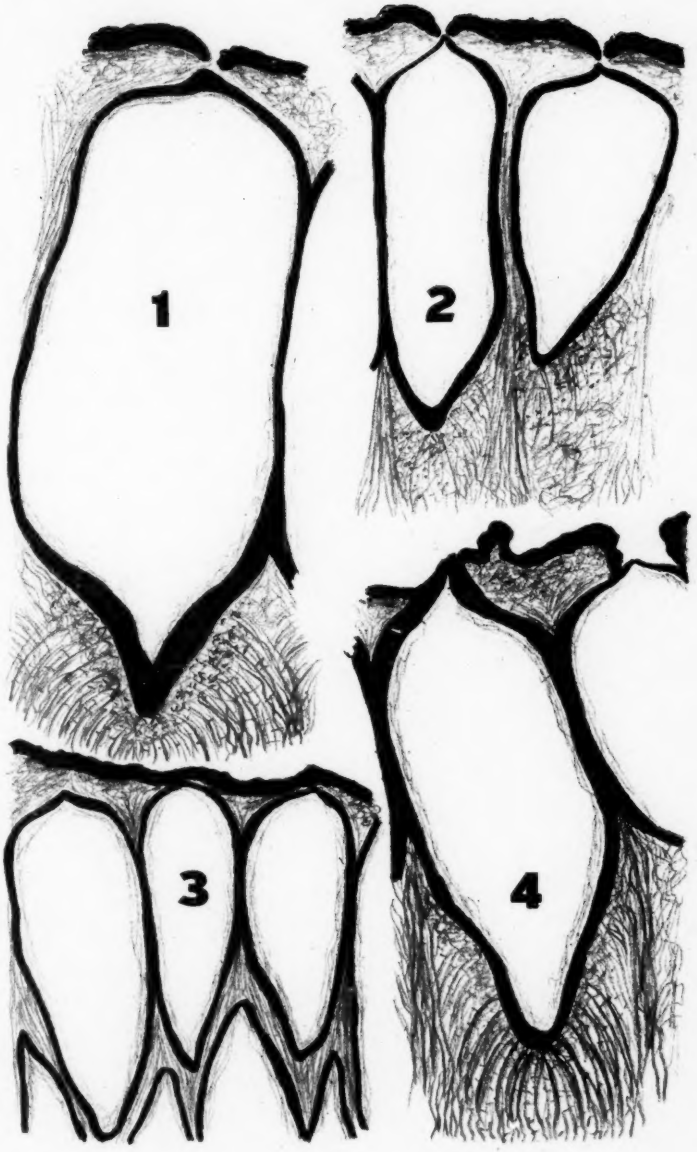
The drawings of the perithecia were all made with the aid of the camera lucida and are drawn to the same magnification.

Fig. 1. *Daldinia loculata*, from material collected in Iowa (Ia 1352).

Fig. 2. *Daldinia loculata*, from material collected in Ohio (Ia). Note the difference within the species of perithecial size and shape.

Fig. 3. *Daldinia grande*, from type material. In this species the perithecia are usually polystichous.

Fig. 4. *Daldinia occidentale*, from material collected by A. S. Rhoads, at Metaline Falls, Washington (PDS). Note that the ostiole penetrates through the ectostroma.



CHILD—THE GENUS DALDINIA

Explanation of Plate

PLATE 33

The drawings of the perithecia were all made by the aid of a camera lucida and are all drawn to the same magnification.

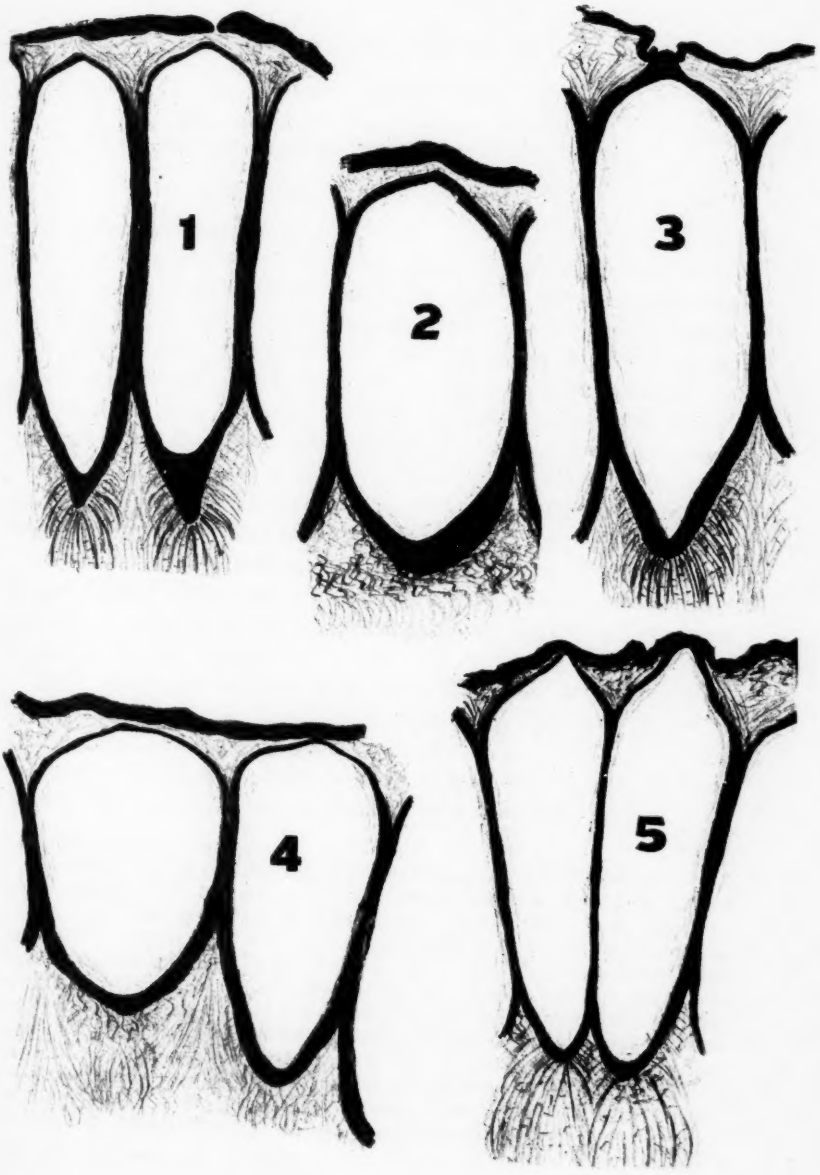
Fig. 1. *Daldinia concentrica*, from material collected in Missouri by Marion Child.

Fig. 2. *Daldinia vernicosa*, from material collected at Fort Riley, Kansas, by Brenckle.

Fig. 3. *Daldinia Eschscholzii*, from material collected in Argentina.

Fig. 4. *Daldinia simulans*, from type material.

Fig. 5. *Daldinia clavata*, from material collected at Matto Grosso, Brazil, by G. O. Malme (St).



CHILD—THE GENUS DALDINIA

STUDIES IN THE UMBELLIFERAE. IV[1]

MILDRED E. MATHIAS

Research Associate, New York Botanical Garden
Formerly Research Assistant, Missouri Botanical Garden

A New Species of Cogswellia

Cogswellia minima,[2] n. sp.

Plants acaulescent, 1–3.5 cm. high, glabrous or scabrous-puberulent; leaves narrowly oblong in general outline, excluding the petiole, 0.5–2.5 cm. long, about 1 cm. broad, simply pinnate with 4–6 pairs of acute, distinct, entire leaflets, 2–10 mm. long, 1–1.5 mm. broad, petiole 1–6 mm. long; peduncles equalling or slightly exceeding the leaves, 1–3.5 cm. long, umbels few-rayed, rays unequal, 3–10 mm. long, pedicels 1–2 mm. long, involucre usually absent, rarely one bract present, involucel subdimidiate, of several more or less distinct, narrow, acute, foliaceous, somewhat scarious-margined bracts, shorter than the yellow flowers; fruit glabrous, oblong in general outline, 4–7 mm. long, 3–4 mm. broad, lateral wings well-developed, dorsal surface prominently ribbed, oil tubes more or less obsolete, strengthening cells present at the base of the wings.

Type specimen: *Mathias 670*, near the hotel, dry slopes bordering Bryce Canyon, Utah, 8600 ft. alt., 18 July 1929 (TYPE in the Missouri Botanical Garden Herbarium).

Distribution: known only from the type locality and the Panguitch Plateau above Cedar Breaks, southwestern Utah.

[1] Issued November 15, 1932.

[2] **Cogswellia minima** Mathias, nov. sp.—Planta acaulis, 1–3.5 cm. alta, glabra vel scabro-puberula; foliis anguste oblongis, petiolis excludentibus, 0.5–2.5 cm. longis, circiter 1 cm. latis, simpliciter pinnatis, foliolis oppositis, 8–12, acutis, distinctis, integris, 2–10 mm. longis, 1–1.5 mm. latis; petiolis 1–6 mm. longis; pedunculis foliis aequalibus vel longioribus, 1–3.5 cm. longis, umbellis pauciradiatis, radiis inaequalibus, 3–10 mm. longis, pedicellis 1–2 mm. longis; involucro plerumque nullo; involucellis subdimidiatis, bracteis pluribus, plus minusve distinctis, angustis, acutis, foliaceis, marginibus subscariosis; floribus flavis brevioribus; fructibus glabris, oblongis, 4–7 mm. longis, 3–4 mm. latis, alis lateralibus conspicuis, jugis dorsalibus prominentibus, vittis plus minusve obsoletis, cellis firmantibus ad basem alarum.—*Mathias 670*, near the hotel, dry slopes bordering Bryce Canyon, Utah, 8600 ft. alt., 18 July, 1929 (TYPE in the Missouri Botanical Garden Herbarium).

Specimens examined:

Utah: Panguitch, 18 July 1920, *M. E. Jones* (P[3] 117944); Cedar Breaks, 17 July 1922, *M. E. Jones* (P 117313); near the hotel, dry slopes bordering Bryce Canyon, 8600 ft. alt., 18 July 1929, *Mathias 670* (M type); on the edge of the Breaks, between the hotel and the camp ground, Cedar Breaks, near Cedar City, Iron Co., about 10,000 ft. alt., 19 July 1929, *Mathias 734* (M).

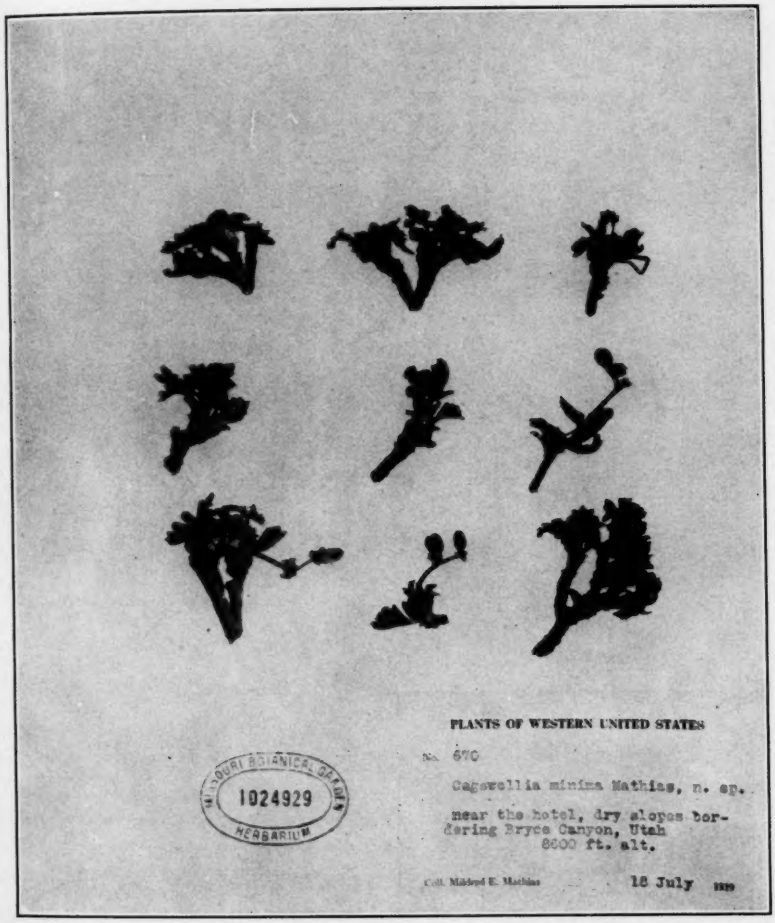
This species is characterized by its dwarf size, the short leaflets, and the short pedicels. It is most closely related to those species of *Cogswellia* referred by Coulter and Rose to the genus *Cynomarathrum* but may be readily distinguished from these species, especially from *Cogswellia Nuttallii* (Gray) Jones and its varieties from northern Nevada and Utah, by the three characters mentioned above and by its distribution in southwestern Utah.

[3] P = Herbarium of Pomona College; M = Missouri Botanical Garden Herbarium.

Explanation of Plate

PLATE 34

Cogswellia minima Mathias. From the type specimen, *Mathias 670*, in the Missouri Botanical Garden Herbarium.



MATHIAS—A NEW SPECIES OF COGSWELLIA

AN APPARATUS FOR THE DETERMINATION OF CARBON DIOXIDE PRODUCTION IN PHYSIOLOGICAL PLANT STUDIES

F. LYLE WYND

Assistant in Botany, Henry Shaw School of Botany of Washington University

From time to time, physiologists have proposed various methods for determining the amount of carbon dioxide evolved in physiological plant studies. These methods vary greatly in the apparatus used, conditions to which they are applicable, and degree of accuracy obtainable.

In the course of investigating the respiratory rate of plants in water culture, a large number of the published methods and apparatus was tested. It was found that liquid absorbents gave incomplete absorption unless the apparatus allowed the bubble to be broken at least three times during its passage through the liquid. In order to accomplish this, it was necessary to use an amount of absorbent which rendered the titration of small differences inaccurate, or to use special units of absorptive apparatus from which the absorbent could only inconveniently be completely removed for each titration.

In view of the inconvenience of using liquid absorbents the present apparatus was designed and it has proved so satisfactory that it was thought to be worthy of being brought to the attention of other workers. The general plan is an adaptation of the widely used method for determining carbon by combustion. The thorough absorption of carbon dioxide has been amply verified by many analysts.

The bell jar *H* is of any convenient size and rests on the glass plate *I*, the contact with which is sealed air tight with vaseline. Air enters the chamber through the large test-tube *D* (32 x 200 mm.) which is filled with soda lime or Ascarite, and is withdrawn through the larger test-tube *J* (38 x 300 mm.) which contains concentrated sulphuric acid. The Folin ammonia tube *K* is especially efficient in breaking up the air bubbles, insuring a more complete drying of the air stream. Tube *L* is of small size and contains phosphorous pentoxide. The absorption bulb

found most convenient was the Fleming or Fleming-Martin type and is designated *P*. The lower chamber contains Ascarite, and the upper, phosphorous pentoxide. The small test-tube *R* also contains phosphorous pentoxide. The air passes out through the suction line *E*. Tower *B* contains calcium chloride, and the jar *A* serves as a safety chamber to prevent water from entering the apparatus through the water pump which attaches to it. The

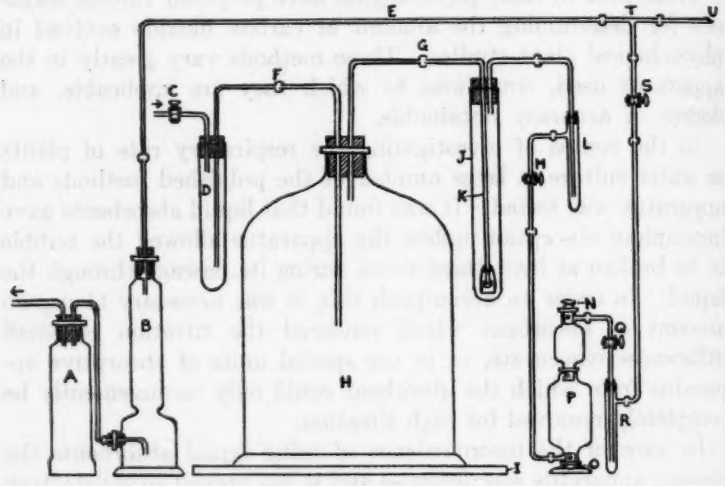


Figure 1

T-tube, *T*, allows several similar units to be operated simultaneously on the same suction line. The tubes *D*, *J*, *L*, and *R* are held in position by clamps.

In carrying out the determination, the rubber tube-connections *F* and *G* are disconnected, which enables the object of study to be placed beneath the bell jar. Stopcock *S* is then closed, and the suction turned on. By slowly opening *S* and counting the bubbles passing through the sulphuric acid tube *J*, the rate of flow may be controlled. The stopcock *S* is used to control the rate of flow rather than *M* or *Q*, because it can be adjusted and not altered. Stopcock *C* is closed when the apparatus is not in use.

Before disconnecting the Fleming bulb for weighing, stopcocks *M* and *Q* are closed to protect the phosphorous pentoxide in tubes *L* and *R* from contact with the atmosphere. The Fleming bulb is itself sealed by turning the top *N* and the base *O*. It is then disconnected and weighed on the analytical balance.

Owing to the impossibility of controlling accurately the temperature of the respiration chamber, for comparative results several units must be run simultaneously. Five units have been found to work very satisfactorily, attached to a single suction line.

The use of Ascarite as the carbon-dioxide absorbent is to be preferred over soda-lime, since its change in color as the carbon dioxide is absorbed indicates when renewal is necessary. A large number of determinations may be made with a single charge.

The drying agent must be more efficient than concentrated sulphuric acid and must be inserted in both sides of the Fleming bulb to establish proper equilibrium. Either phosphorous pentoxide or "Hydralo" (Al_2O_3) is satisfactory. In any case, a plug of glass wool should be inserted above it to prevent the air current from carrying away dust-like particles.

The accuracy of the determination is limited by the amount of carbon dioxide in the apparatus at the beginning and by the sensitivity of the balance used in weighing. The filled Fleming tube weighs about 150 grams, but this weight allows a balance sensitivity sufficiently great for most experiments.

A greater degree of accuracy may be obtained in weighing the Fleming bulb if it is allowed to stand on the balance twenty minutes, and the base opened and quickly closed before its weight is determined. A similar but unfilled bulb on the pan with the weights reduces the error involved by the presence of different amounts of moisture adsorbed on the surface at different times of the day.

The above-described apparatus is particularly adapted for measuring the carbon dioxide output of fruits, soils, and plants in water culture. In the latter case, the nutrient solution should be renewed just before the determination is made in order to reduce the possibility of introducing an error by the activity of micro-organisms.

A NEW SPECIES OF PARMELIA FROM TEXAS[1]

JOHN ADAM MOORE

Formerly Rufus J. Lackland Research Fellow in the Henry Shaw School of Botany of Washington University

Among the interesting lichens collected by Mr. Julian A. Steyermark and the writer in the mountains of western Texas was one *Parmelia* which appears to be unique. This is now described as:—

Parmelia incorrupta J. A. Moore, sp. nov.

Thallus 4–7 cm. latus, profunde lobato-incisus, lobi lati irregulariter undulato-crenulati non ciliati. Thallus juventate laevigatus vel maturitate reticulati-rugosus, punctis albis, sorediis et isidiis destitutus. Color flavulo-viridis glaucescens, subtus centrum nigrum asperum paucis rhizinis concoloribus, lobi juventate laevigatae fulvi nudi.

Cortex superior 20 μ altus, pseudoparenchymaticus, parte exteriore dilute fuscescens, ceterum hyalinus. Medulla alba, 100–125 μ alta, hyphae adspersa. Gonidia diam. 8 μ. Cortex inferior pachydermaticus fusconigrescens, 15 μ altus.

Apothecia numerosa, sessilia, rotunda vel angulosa, diam. 2–10 mm., margine eroso non ciliato. Gonidia sub excipulo et infra hymenio. Excipulum decolor, 70 μ altum, hypothecium 100 μ altum. Discus concavus badius laevigatus.

Hymenium superne fuscescens, subtus decolor, 60–75 μ altum. Paraphyses graciles, apices incrassata, libera. Asci ovati-clavati, octospori, 36 μ longi, 18 μ crassi. Sporae biserialiter dispositae, ellipsoideae, 12 μ longae.

Conceptacula pycnidiorum numerosa immersa, subglobosa, diam. 180 μ.

React. Omnes partes non mutantur. KOH −, CaCl −; KOH(CaCl) −.

TYPE COLLECTION: On bark of *Pinus flexilis* James, ridge above McKittrick Canyon, Guadalupe Mountains, Culberson Co., Texas. July 17, 1931. *J. A. Moore & J. A. Steyermark 3490.* TYPE in Herbarium at the Missouri Botanical Garden.

[1] Issued November 15, 1932.

Additional material seen:

Texas: on oak bark, Boot Spring, Chisos Mountains, Brewster Co., *J. A. Moore & J. A. Steyermark 3220* (MBG[1]); Ft. Bliss, *Mrs. Jos. Clemens 11358* (MBG).

Mexico: Mexico, *E. Palmer 1878* (MBG); oak trees, Orizaba, alt. 10,000 ft., *J. G. Smith 34* (MBG).

This new species belongs in the subgenus *Euparmelia*, section *Amphigymnia*, group *Subflavescentes*. It is most closely related to *Parmelia caperata* (L.) Ach., from which it differs in having a larger thallus, the upper surface being white punctate. The new species shows no color reactions with KOH and CaCl even after the reagents have been applied for several hours; while the thallus of *P. caperata* quickly responds to KOH by turning yellow, with CaCl no reaction, but with both reagents the thallus quickly becomes yellow below.

With the evident lack of chemical reaction of the new species in mind, the specific epithet *incorrupta* was chosen.

[1] MBG = Missouri Botanical Garden Herbarium.

SOME EFFECTS OF RADIATIONS FROM A MERCURY VAPOR ARC IN QUARTZ UPON ENZYMES[1]

HARRY JAMES FULLER

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I. Review of Previous Experimentation

Numerous experiments have been performed with the object of determining the effects of radiations from mercury and carbon arcs and from the sun upon enzymes *in vitro*. The results have usually shown agreement, in one feature at least—that light sources which emit ultra-violet rays induce partial or complete inactivation of the irradiated enzyme solutions, depending upon the intensity or irradiation and the duration of exposure. In most of these experiments standardization of certain physical conditions of the procedures has been neglected, with the consequence that certain results have been at variance. The two features which have been most neglected are these: (1) Quantitative measurements of the energy emission of the light sources; (2) The determination of comparative effects of different portions of the spectrum upon the enzymes in question. In most of the published works, energy measurements have been omitted completely, so that it is impossible to review various experiments upon a comparative basis. Moreover, the destructive effects of the radiations have been assigned almost exclusively to the ultra-violet portion of the spectrum, with complete disregard for possible effects of other wave-lengths. It has been fairly well established that the visible rays exert little or no effect, but the influence of infra-red radiations which form a large percentage of the energy emission of arcs has been overlooked. Some of the more recent experimenters have irradiated enzyme solutions in quartz vessels immersed in water baths, but they have made no

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studies upon the relative effects of the infra-red and ultra-violet wave-lengths.

In this review of literature, only those experiments which involve ultra-violet radiations will be summarized. It is both irrelevant and unnecessary to dwell upon the effects upon enzymes of other types of radiation: X-rays, radium emanations, etc.

Probably the first biologists to study the effects of light upon enzymes were Downes and Blunt ('79), who found that zymase was inactivated in quartz tubes by the full solar spectrum in the presence of oxygen; they concluded that the injurious effect was due chiefly to the blue-violet portion of the spectrum and that the inactivation process was an oxidation reaction. Fermi and Pernossi ('94), in a study of a number of environmental factors upon enzymes, found that pepsin and trypsin in aqueous solutions are attenuated by long exposure to sunlight, that diastase and ptyalin are but slightly injured by such exposure, and that bacteria exposed to sunlight lost considerable of their proteolytic activity. Green ('97) studied in great detail the action of sunlight and light from an electric arc upon diastase in leaves and in aqueous extracts; he found that both in the tissues and extracts the diastase suffered a considerable loss in activity, but that such injury did not occur when ultra-violet rays were removed by glass filters. In fact, when such filters were used, Green found that an increase in diastatic activity often occurred. He likewise showed that the proteins and perhaps the chlorophyll in leaf cells exert a protective influence for the enzymes against the abiotic ultra-violet rays. Emmerling ('01) contributed further to the study of the effects of sunlight upon enzymes; he found that invertase, maltase, lactase, amylase, and rennet suffered various degrees of attenuation, but that pepsin, trypsin, and emulsin were apparently unaffected. Von Tappeiner ('03) studied the effects of sunlight upon enzymes with and without the addition of fluorescing substances such as Magdala red, chinolin red, and eosin; he found in the case of diastase, invertase, and papayotin that little or no injury occurred when exposure was made without a fluorescing substance, but that when such a material was added, even in so dilute a concentration as 1: 1,000,000, definite attenuation of enzyme activity resulted.

The Schmidt-Nielsens ('04, '08) exposed solutions of chymosin, chymosinogen, and antichymosin of blood serum for short periods to a carbon arc and found that in all cases an exposure of fifteen minutes was sufficient to produce as much as 95 per cent inactivation; when the ultra-violet spectrum was removed by filters no inactivation occurred, and they concluded that the ultra-violet rays were responsible for the damage. Jodlbauer and von Tappeiner ('06) exposed solutions of invertase to sunlight with and without the ultra-violet rays, and found that sunlight without ultra-violet was capable of producing a small amount of injury, which was increased to 80 per cent in ten minutes if a small amount of a fluorescing substance was added. The same authors later ('06a) rayed solutions of invertase with a carbon arc in a water bath to exclude infra-red radiations and found a definite injury when oxygen was present; from this, they concluded that the process was an oxidation reaction. Dreyer and Hanssen ('07) found that the radiations from a mercury vapor arc weakened the enzymes rennet, trypsin, and papayotin; they believed that the ultra-violet radiation was chiefly responsible for this injury, since a glass plate inserted between the arc and the enzymes prevented attenuation. The Schmidt-Nielsens ('08) rayed commercial solutions of rennet in a water bath beneath a mercury vapor arc and found a destruction of activity proportional to the length of irradiation, to the energy given off, and to the dilution, the greater dilutions of the enzyme suffering a greater degree of injury. With the use of special filters they determined that 93 per cent of the injury was attributable to the wave-lengths 200–250 mμ, 4 per cent to 250–313 mμ, and 3 per cent to the visible spectrum. They supposed the destruction process to be monomolecular and to be more or less independent of temperature.

Pougnet ('10) found that in the cumarin plant the characteristic odor is formed after irradiation. This process is dependent upon the activity of enzymes which were thus shown to be uninjured by the radiations. Pougnet ('11) found a similar condition in the production of odor in vanilla pods. Stassano and Lematte ('11) exposed suspensions of bacteria to a mercury vapor arc beneath a thin film of water to remove heat rays and found that

bacteria killed by such exposure produced agglutination which was only slightly less pronounced than that caused by living bacteria; hence they concluded that the inactivation of enzymes contributed little if any to the killing of bacteria by ultra-violet rays. Chauchard and Mazoué ('11) rayed solutions of malt amylase and yeast invertase with a quartz mercury vapor arc in separate and in mixed solutions and found that the amylase at the end of one hour's exposure was only 10 per cent as active as unrayed solutions, but that invertase had lost only 45 per cent of its activity. They concluded that it was possible to destroy amylase completely and have some active invertase left if a mixture of the two were rayed for a sufficient period of time. Agulhon ('12) studied the effects of radiations from a mercury arc upon sucrase, malt amylase, pancreatic amylase, pepsin, emulsin, and rennet, and reported that all of these enzymes suffered some degree of attenuation from wave-lengths shut out by glass but transmitted by quartz—in other words, the injury was caused by ultra-violet rays, with the visible rays contributing practically nothing to this effect. Burge ('17), in attempting to discover the mechanism by which ultra-violet rays kill protoplasm, found that such organisms as *Bacillus liquefaciens*, *B. prodigiosus*, etc., could be killed by ultra-violet rays without any apparent decrease in the power of their extracted enzymes to liquefy gelatin.

In 1923, Ludwig Pincussen, at the City Hospital of Urban, Berlin, began the publication of a series of papers entitled "Fermente und Licht," which reported a number of experiments conducted by him and his associates upon that subject. The first paper ('23) described the effects of sunlight and of a quartz mercury arc upon solutions of malt diastase as follows: 1. Sunlight had little or no effect upon the enzymes. 2. The mercury arc produced strong inactivation. In the summary of his paper, Pincussen mentions certain laws which seem characteristic of the action of abiotic radiations upon enzymes: "Die Wirkung des Lichtes auf die Malzdiastase ist äbhangig von der Verdünnung, von verschiedenen Begleitkörpern und vor allem von der Reaktion. Die grösste Lichtschädigung erfolgt bei einer Reaktion die als die optimale für die Fermentwirkung anzusehen ist. Es

scheint demnach, dass das Fermentmolekül bei seiner optimalen Wirkungsreaktion besonders labil gegenüber der Lichtenergie ist." Furthermore, Pincussen writes "Es scheint dass in gewissen Grenzen die Temperatur ohne Einfluss auf eine mögliche Lichtschädigung ist," thus omitting possible effects of the infra-red radiation from the arc upon the enzyme solutions.

Pincussen and Kato ('23) exposed solutions of soy-bean urease to sunlight and to the radiations from a quartz mercury arc and found that both strong sunlight and the arc radiations produced injury which was greatest at the optimum pH of the enzyme. Essentially the same results were found in a somewhat more extensive paper by Pincussen and Kato published later ('23a). Pincussen and di Renzo ('24) continued the work on diastase in various concentrations; they found that the injury produced by a mercury vapor arc decreased with increasing concentration of the enzyme in solution and that the inactivation reaction is monomolecular. Pincussen ('24) rayed malt and Taka-diastase and found the Taka-diastase somewhat more easily inactivated by ultra-violet than the malt-diastase. He discovered that when certain salts were added to the enzyme solutions, the degree of injury caused by the ultra-violet rays was noticeably decreased. He ('24a) continued his studies upon the protective action of salts and found that iodine salts particularly were instrumental in causing protection against ultra-violet rays. With Klissiunis ('24) he studied further the protective action of iodine salts, potassium and sodium iodides, and in addition the iodides of alkali earths, lithium iodide, rubidium iodide, etc., upon solutions of trypsin. They found that the addition of any of these salts decreased considerably the injury produced by light in the enzyme solutions. Pincussen and Seligsohn ('26) rayed solutions of blood catalase at 10 cm. from a mercury arc (with no protective measures against infra-red) and found that inactivation followed the laws for other enzymes: the proportionality of inactivation to dilution and optimum pH, and the protective action of salts, reported previously for diastase and trypsin. Pincussen ('26) reported additional experiments upon diastase, in which he described a "reactivation" effect in the case of purified malt diastase. When diastase solutions which had been injured in

varying degrees by exposure to a mercury arc were mixed with unrayed solutions, the mixtures showed greater diastatic activity than the sum of the activities of the separate solutions. This reactivation is the more intense the greater is the injury to the rayed solutions; when the injury is slight there is little or no reactivation; further, the reactivation is more striking the greater are the quantities of rayed diastase in the mixture.

Pincussen and Kumanomidoh ('28), in further work upon the addition of salts in relation to light inactivation, discovered a protective action of the chlorides of K, Ca, Mg, Li, and Pb. The order of protectivity varied with the different diastases used —salivary, malt, and Taka—and also with the concentrations of the salts and of the enzyme solutions. In general, the potassium salts seemed to offer the least protection, the calcium salts the greatest. Pincussen and Uehara ('28) rayed solutions of pepsin in a water bath with a mercury vapor arc and found definite inactivation which was greatest at pH 1.15, which is near the optimum for peptic activity. At pH 1.93 and pH 2.28 there was no significant difference between the activities of rayed and unrayed pepsin solutions. Pincussen and Hayashi ('28) used serum lipase from rabbits and guinea pigs, which was exposed at twenty centimeters from a mercury arc (again with no consideration of infra-red) and which suffered definite injury chiefly at acid pH values. When accompanying proteins were removed by precipitation with ammonium sulphate the injury was greater. The injury was practically nil at alkaline pH values. Pincussen and Kambayashi ('28) studied the effects of sensitizing agents, particularly eosin, upon the inactivation of Taka-diastase by ultraviolet and found that the addition of the agent in no case increased the degree of injury produced by a mercury arc, a result contradictory to earlier findings, and that with increasing concentration of the sensitizing agent there was a decrease in the amount of injury produced by light. In all cases the greatest inactivation occurred at the optimum pH, with or without the addition of the fluorescing agent. Pincussen and Oya ('29) studied the effects of light from a mercury arc upon Taka-diastase solutions in water baths at different temperatures. They found greater inactivation at higher temperatures (50° C.) than at

lower ones (16°). They found no reactivation phenomena. Pincussen and Oya ('29a) rayed solutions of Taka-diastase with a mercury arc and studied the lecithin-hydrolyzing power of the enzymes lecithase and phosphatase, which are invariably present in the diastase preparation under such treatment. They found a decrease in the activity of these two enzymes; the above-mentioned laws held true here again. They ('29b) found a great degree of inactivation of milk aldehydrase after an irradiation of one hour with a mercury vapor arc in the presence of oxygen. Pincussen and Roman ('30) found that the succinodehydrogenase of horse muscle was strongly injured in the pH region 6.0–8.0 by a fifteen-minute exposure to a quartz mercury arc; a lesser injury was produced by one hour's exposure to visible light.

Thompson and Hussey ('25–'26) rayed aqueous solutions of pepsin in a water bath above a mercury vapor arc and found definite inactivation of the enzyme. They concluded that the process was that of a monomolecular chemical change. They also decided, from that fact that .5 cm. of water separated the enzyme tubes from the arc, that "it would seem that the effective radiations are those in the ultra-violet region of the spectrum." In view of the fact that .5 cm. of water removes only a portion of the infra-red radiation, this assumption might be questioned.

Tauber ('30) found that exposure to direct sunlight does not injure urease; if, however, eosin is added, definite injury occurs. The radiations from a quartz mercury arc he found likewise to be inhibitory to the activity of urease; eosin increased the injurious effect. Thompson and Hussey ('31) rayed amylase solutions in a water bath with a mercury vapor arc and found inactivation which followed the course of a monomolecular radiochemical change and which they attributed solely to the ultra-violet wavelengths in the arc spectrum. Pace ('31) studied the effects of radiations from a mercury arc upon solutions of trypsin and enterokinase which had been previously partially inactivated by heat; with irradiation, only further inactivation occurred; with visible light only, there was no further effect upon the enzymes. Pace made no provisions in his work for removing infra-red radiation.

Thus the uncontrolled features of these experiments, empha-

sized in an opening paragraph, are made obvious: (1) The failure to consider the energy output of the light source; (2) The neglect of the possibility of enzyme injury by infra-red radiation. As mentioned in the review of literature, a few experimenters have taken precautions for the removal of infra-red but in no work so far has a comparative study of the effects of ultra-violet and infra-red been completed; (3) The almost complete lack of study of the effects of ultra-violet radiation upon enzymes *in vivo;* too often the results of test-tube experiments have been utilized to explain the effects of radiations upon organisms without attempting to study the effects upon enzymes in tissues.

II. Statement of the Problem

It was the object of the experiments reported in this paper, first, to study the effects of radiations from a quartz mercury vapor arc upon two commonly used enzyme preparations, Taka-diastase and Difco invertase, with respect to the comparative effects of infra-red and ultra-violet portions of the spectrum upon them, with careful measurements of the energy output of the irradiation sources; second, to study the effects of the full ultra-violet spectrum, plus the visible spectrum, upon enzymes in plant tissues, with a view to determining whether or not the injury to these tissues is caused by injury to enzymes.

These two groups of experiments will be designated respectively as Series I and Series II.

III. Experimental Methods and Results

SERIES I

In this group of experiments, Taka-diastase and Difco invertase were used. In both cases, aqueous solutions of the enzymes were rayed in 2-mm. layers in open Petri dishes at 10 inches from a quartz mercury vapor arc, a Burdick lamp operated at 75 volts and 6 amperes. Experiments with infra-red rays present and with infra-red rays excluded were conducted in parallel. For the removal of the infra-red rays from the spectrum, a quartz water cell, consisting of a chamber with a 6-inch square of quartz 2 mm. thick for the bottom and a 1.5-cm. layer of water, was inserted midway between the arc and the enzyme solutions. Such a filter

removed the greater part of the infra-red radiation and transmitted without appreciable diminution of energy the ultra-violet wave-lengths. Hence, with the water cell in place it was possible to study the effects of the visible and ultra-violet spectra together, without the infra-red; since it has been conclusively demonstrated that the visible spectrum has a negligible effect upon enzymes, the results obtained with the water cell may be attributed exclusively to the ultra-violet rays. When the water cell is not inserted the combined effect of ultra-violet and of infra-red is obtained, from which by simple subtraction one can determine the extent to which infra-red produces injury and thus draw a comparison with the effects of ultra-violet alone, unless the combination of infra-red and ultra-violet produces a special effect.

Intensity measurements were made by means of a Leeds & Northrup type P reflecting galvanometer No. 2239 with a sensitivity of .7 microamperes, and two Cenco linear thermopiles arranged in parallel. A carbon filament incandescent lamp from the United States Bureau of Standards, standardized to give a radiation of 86.2×10^{-8} watts per sq. mm. of receiving surface at 2 meters when lighted at .4 amperes and 99.5 volts, was used as a basis for computing the radiant energy given off by the arc. The intensity measurements for Series I are as follows:

At a distance of 10 inches from the arc, with the quartz water cell in place: 5025.0×10^{-8} watts per sq. mm., or 1202.15×10^{-8} gm. calories per second per sq. mm. Without the water cell, at the same distance: $45,225.0 \times 10^{-8}$ watts per sq. mm. or $10,826.17 \times 10^{-8}$ gm. calories per second per sq. mm. It is evident that at this distance from the arc the energy falling upon a unit of area is composed of about 12 per cent ultra-violet and visible and about 88 per cent infra-red.

1. Taka-diastase.—Taka-diastase was rayed in .5 per cent aqueous solution in 10-cc. portions with and without the water cell before the arc, as described above. In all cases the period of exposure was 30 minutes. At the end of the irradiation period, distilled water was added to make the solutions up to 10 cc. to make up for evaporation during the irradiation. Then the activity of the rayed sets and of the unrayed (controls) was de-

termined by Appleman's ('11) modification of the Wohlgemuth ('08) iodine method for diastase, which is described below.

Three sets of 10 test-tubes each were arranged in test-tube racks and were designated *a*, *b*, and *c*, which referred respectively to the solutions unrayed, rayed with the water cell, and rayed without the water cell. In each set the tubes were numbered in units from 1 through 10. Ten cc. of a 2 per cent Lintner soluble starch solution were placed in each tube, and the 3 sets were immersed in an ice bath until the temperature of the starch solution in the tubes was the same as that of the bath; this was done to prevent enzyme action until all tubes were inoculated and could be set in the incubator. Then, in each set 1 cc. of the enzyme solution was placed in the tube numbered 10, .9 cc. in tube 9, .8 cc. in tube 8, and so on, down through tube 1, which received .1 cc. of the enzyme solution.

After the enzyme solutions had been added to the proper tubes the tubes were all set away in an incubator adjusted to 39° C., where they remained for 45 minutes. Following this incubation period, the tubes were again immersed in an ice bath until the temperature of the enzyme-substrate mixtures was the same as that of the bath, thus preventing further enzyme action. Then 2 cc. of the mixture were pipetted out from each tube into a glass vial bearing a number corresponding to that of the tube, 5 cc. of distilled water and 2 drops of iodine-potassium iodide solution (Meyer's) were added, and then color changes in the tubes in the 3 sets were noted. A yellow color indicated complete hydrolysis of starch; a reddish-yellow, a complete disappearance of starch but the presence of some dextrine; a red-violet, not quite complete disappearance of starch; and a definite violet color, the presence of considerable quantities of starch. The first tube in the descending series which showed a violet tinge was the index for comparing diastatic activity, since it represented the lowest limit of enzyme activity. The activity of the enzyme is expressed as the number of cc. of the starch solution which 1 cc. of the enzyme solution could hydrolyze during the incubation. For example, if tube 4, containing .4 cc. of enzyme solution, had the first tinge of violet in the descending series (that is, has practically entirely hydrolyzed the starch in that tube), then 1 cc. of

the enzyme solution under the same conditions would hydrolyze $\frac{1.0}{.4}$ or 2.5 times the same quantity of starch solution. If 10 cc. of starch solution is used as the substratum, then the quantity of solution which would be hydrolyzed by 1 cc. of the enzyme solution would be 10×2.5 or 25 cc. This figure is taken as the diastatic power of that enzyme under the specified conditions. The conditions and the diastatic power are usually expressed thus:

$$D\frac{T}{t} = N$$

wherein D is the diastatic power, T the temperature of incubation, t the duration of incubation and N the number of cc. of starch solution hydrolyzed by one cc. of the enzyme solution.

The Wohlgemuth method is perhaps not as fine as the copper method of measuring amyloclastic activity, but as Sherman, Kendall, and Clark ('10) write: "Although it is not quite as accurate as the gravimetric copper method, it is easier to perform and has the theoretical advantage of marking the completion of a fairly definite step in the digestive process, whereas the copper reduction method measures the amount of a substance or substances produced by successive steps through intermediate products which are but imperfectly known."

TABLE I

TAKA-DIASTASE

(T, 39°, t, 45 min.)

Experiment number	Set A Unrayed D	Set B Rayed with water cell D	Set C Rayed without water cell D
1	100.0	14.3	10.0
2	100.0	16.7	10.0
3	100.0	16.7	10.0
4	100.0	16.7	10.0
Average	100.0	16.1	10.0

The results of the Taka-diastase experiments are presented in table I.

From the table it is evident: (1). That considerable injury occurs in Taka-diastase with the water cell in place, and that such injury is caused by ultra-violet radiation; (2). That greater injury occurs when the water cell is removed; that is, the infra-red exerts an appreciable injurious effect in addition to that of the ultra-violet rays.

2. Difco invertase.—The Difco standardized invertase solution was diluted in equal parts with distilled water and then rayed in open Petri dishes under exactly the same conditions as those employed in the experiments upon Taka-diastase. The experimental sets were designated in the same way: A, the unrayed set; B, the set rayed with the water cell; C, the set rayed without the water cell.

After the irradiation, 20 cc. of the enzyme solution were added to 150 cc. of 20 per cent sucrose solution, previously adjusted to pH 4.6 with .1 N sodium acetate. The activity of the invertase solutions was determined by the polarimetric method as described by Waksman and Davison ('26). The rotation of each invertase-sucrose solution was determined immediately after the addition of the invertase and then from time to time thereafter to deter-

TABLE II

DIFCO INVERTASE

(t, time in minutes required to reach end point)

Experiment number	Set A Unrayed t	Set B Rayed with water cell t	Set C Rayed without water cell t
1	30.0	35.7	41.1
2	32.0	39.0	46.0
3	30.0	36.0	42.0
Average	30.66	36.90	43.03

mine the time required to reach the end point. During this interval the solutions were kept in an incubator at 36° C. The periods of time required to bring the solutions to the same end point were taken as the indices of enzymatic activity (Waksman and Davison, '26, p. 177). The results of the invertase experiments are presented in table II. The table shows that the same

results which obtained in the diastase experiments were evident here: there is definite injury caused by the ultra-violet radiation, which is significantly increased when the infra-red rays also are present to act upon the enzyme.

Expressing the value of the unrayed set average as 100, we find the values of sets B and C to be respectively 89.00 and 70.75.

SERIES II

The experiments described in this section were designed to study the effects of the ultra-violet radiations from a mercury vapor arc upon enzymes in plant tissues, with a view of obtaining information concerning the physiologic mechanism by which such radiations damage and kill vegetable tissues. The enzymes studied were amylase, invertase, peptase, and catalase. The plants used were "Bonny Best" tomatoes, red kidney field beans, and a strain of *Fusarium Lini* Bolley, kindly provided by Mr. W. E. Brentzel of the North Dakota Agricultural Experiment Station. As pointed out in the review of literature, the effects of ultra-violet rays upon enzymes *in vivo* have been studied in only two or three cases, and in those (with the exception of Green's work) the studies have been limited to the enzymes of bacteria. So far as the author is aware, this is the first paper in which studies of the effects of ultra-violet rays upon several enzymes in the tissues of multicellular plant forms are reported in detail.

A. BEANS AND TOMATOES

The bean and tomato plants were grown in individual 4-inch pots to a height of about 20 cm. when irradiation was begun. The plants were rayed 3 minutes daily for 7 days at a distance of 50 cm. from the arc. A quartz water cell containing 1.5 cm. of distilled water was placed a short distance below the arc to remove the infra-red rays from the radiations which reached the plants. Intensity measurements, made as described in Series I, showed 1256.25×10^{-8} watts per sq. mm. or 302×10^{-8} gm. calories per sq. mm. per second.

In both the beans and tomatoes the irradiation produced serious damage in the plants. The upper epidermises of the leaves were badly burned and the leaves showed the bronzing and

curling typical of ultra-violet injury. At the end of the 7-day period, the enzyme determinations were made. In all cases the measurements of activity were made on fresh tissue.

1. Amylase.—Ten gms. of fresh leaf tissue, after the removal of petioles, were ground with 10 gms. of quartz sand in a mortar for 3 minutes. Five cc. of distilled water were added to the mixture and the juice was pressed out through two layers of cheesecloth, after which it was centrifuged at 2000 r.p.m. for 4 minutes to remove solid materials from the supernatant liquid. The activity of the amylase in the juice was then determined by the Wohlgemuth method as described in Series I. One cc., .9 cc., .8 cc., and similarly descending quantities of the juice were placed in the properly marked test-tubes, each of which contained 10 cc. of chilled 1 per cent Lintner soluble starch solution. As a preservative, .5 cc. of toluol was added to each tube, after which the tubes were stoppered and set away in an incubator maintained at 39° C. It was found by preliminary trials that incubation periods of 6 hours for beans and 24 hours for the tomatoes were the optima. After the incubation the tubes were again chilled with ice and 2 cc. of liquid from each tube were pipetted off into vials marked to correspond with the test-tubes. Five cc. of distilled water and 2 drops of iodine solution were added to each vial, and color comparisons made. The results of the amylase experiments are presented in table III.

TABLE III

AMYLASE

(T, 39°; t, 6 (beans)—t, 24 (tomatoes))

Experiment number	Beans		Tomatoes	
	Unrayed D	Rayed D	Unrayed D	Rayed D
1	16.6	50.0	16.6	25.0
2	16.6	33.3	16.6	25.0
3	16.6	33.3	16.6	20.0
Average	16.6	38.8	16.6	23.3

Choosing 100 arbitrarily as the value of the activity of the amylase in the averages of the unrayed groups, we find the following comparisons:

Beans—Unrayed, 100.00; rayed, 258.40.
Tomatoes—Unrayed, 100.00; rayed, 155.37.

The figures show conclusively that there is a definite and significant increase in the amyloclastic activity of the juice of tomato and bean plants which have been badly injured by ultra-violet radiation.

2. Invertase.—In these experiments the plant juice was extracted and prepared in the same fashion as in the amylase experiments. The activity of the enzyme was determined by adding 5 cc. of the juice to 100 cc. of 15 per cent sucrose solution and incubating for 12 hours at 39° C. At the end of this period, 10 cc. of the mixture were added to 30 cc. of Fehling's solution which was just at the boiling point, and the mixture was allowed to boil for exactly 3 minutes. The tubes were then cooled and the cuprous oxide precipitate was determined quantitatively by titration with potassium permanganate as follows: The solutions were filtered and the residue of cuprous oxide was dissolved in Bertrand's solution (20 per cent sulphuric acid saturated with ferric sulphate), and this solution was then titrated with twentieth-normal potassium permanganate solution. The number of cc. of the permanganate solution required to give the copper-Bertrand solution a faint permanent violet tinge was taken as the index of enzyme activity.

In the invertase experiments, blank solutions were arranged which contained 5 cc. of the plant juice and 100 cc. of distilled water substituted for the sucrose solution. The copper in these solutions was determined just as in the solutions containing sucrose; in this way the quantity of invert sugars present in the plant extract was determined. This value was then subtracted from that obtained from the enzyme-sugar solution to give the actual amount of reducing sugars formed in the latter as a result of invertase activity.

The results of the invertase experiments are presented in table IV.

Using the value 100 as an expression of invertase activity in the averages of the unrayed sets, we find the activity in sets to be:

Beans—unrayed, 100.00; rayed, 124.29.
Tomatoes—unrayed, 100.00; rayed, 125.99.

TABLE IV

INVERTASE

(cc. of potassium permanganate)

Experiment number	Beans		Tomatoes	
	Unrayed	Rayed	Unrayed	Rayed
1	8.6	11.0	11.5	15.0
2	8.4	9.8	11.2	14.5
3	8.2	9.9	14.1	16.9
Average	8.4	10.23	12.26	15.46

Thus a distinct increase is demonstrated in the activity of invertase in the rayed plants.

3. Peptase.—The term "peptase" is used here as proposed by Fisher ('19) to include those enzymes whose activity is peptoclastic; the term is synonymous with the name "ereptase" proposed by Vines at an earlier date. The method of determining the activity of the peptase was that of Sorensen ('08) as reported by Fisher ('19), by means of which the degree of hydrolysis of the substratum is determined from the number of free hydroxyl groups formed.

The measurements of activity were made as follows:

Ten grams of fresh leaf material, without petioles, were ground vigorously with 10 g. of quartz sand in a mortar for 3 minutes, after which the mixture of pulp and sand was added to a solution of 5 gms. of Witte's peptone in 250 cc. of distilled water. The solution was covered with toluol as a preservative and placed in an incubator at 38° C. for 3 days. At the end of that time, the solutions were filtered rapidly through Buchner filters by vacuum, and the residue was washed with distilled water until the original solution and the washings made up a volume of 400 cc. Forty cc. of the filtrate were removed to a small flask and decolorized by shaking with 1 g. of alumina cream. After standing for 4 minutes, the solution was filtered and the residue washed in distilled water to make a volume of 65 cc. of filtrate. To this were added 15 cc. of a solution of thymolphthalein made up as follows: 50 cc. of 40 per cent formaldehyde, 25 cc. of absolute alcohol, and 10 cc. of thymolphthalein solution (.5 g. in 1000 cc. of 93 per cent alcohol).

The formaldehyde neutralized the free amino groups of the amino acids produced, forming methylene compounds; the carboxyl groups were then determined by titrating with fifth-normal barium hydroxide solution, the thymolphthalein acting as an indicator. The barium hydroxide solution was added to the decolorized filtrate until a distinct blue color developed.

It was necessary to apply corrections to compensate for the amino acids already present in the tissues and in the peptone. For this the tissue was ground as described above and added to 250 cc. of distilled water without peptone. After 3 days of incubation the determination was applied as above, the obtained value representing the relative amino acid content of the tissue. To determine the amino acids originally present in the peptone, a flask containing 250 cc. of 2 per cent peptone solution only was incubated for the usual period of time and then its amino acid content determined by the titration method. These two values—the amino acid content of the tissue and that of the peptone—were subtracted from those obtained from the tissue-peptone mixtures; the resultant figures represented the actual amount of amino acids formed as a result of the hydrolysis of the peptone by the peptase. The amount of the hydrolysis can be calculated as milligrams of nitrogen by multiplying the number of cc. of fifth normal barium hydroxide solution required to produce a blue color by 2.8. The results of the peptase experiments are shown in table v.

TABLE V

PEPTASE

(Milligrams of nitrogen liberated)

Experiment number	Beans		Tomatoes	
	Unrayed	Rayed	Unrayed	Rayed
1	165.0	198.6	103.6	140.0
2	176.4	201.6	123.2	148.4
3	182.0	207.2	112.0	134.4
Average	174.46	202.53	112.60	140.93

Expressed on the basis of 100 as the value of the controls, the following figures for the averages are obtained:

Beans—unrayed, 100.00; rayed, 115.44.
Tomatoes—unrayed, 100.00; rayed, 124.01.

Thus, the peptoclastic activity of the juice of tomato and bean plants which were damaged by ultra-violet rays seems consistently higher than that of unrayed plants.

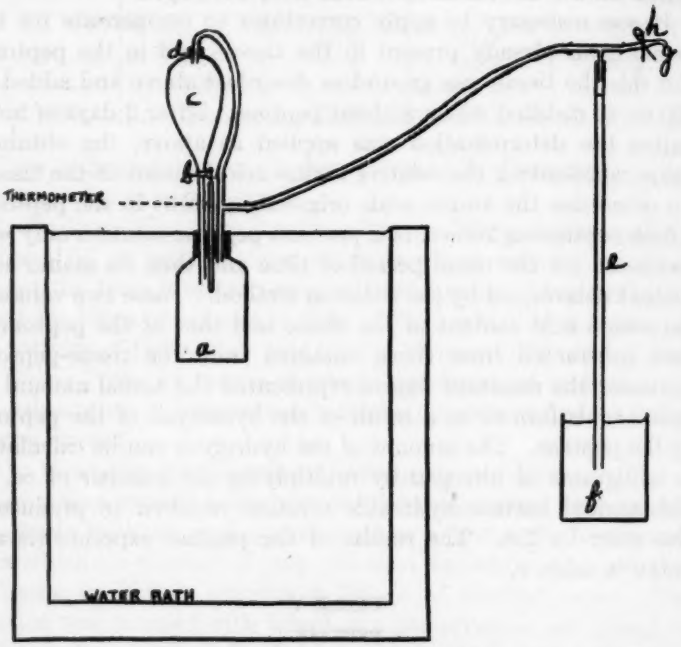

Fig. 1. Apparatus used in determining catalase activity: *a*, reaction bottle; *b*, stopcock; *c*, funnel; *d*, stopper; *e*, burette; *f*, water reservoir; *g*, rubber tube; *h*, stopcock.

4. Catalase.—The catalase activity of the juices of the rayed and unrayed plants was determined by the usual hydrogen peroxide method in a modified Appleman ('10) apparatus.[1] The apparatus used in these experiments differs from that devised by Appleman in that it has but one burette containing a column of water supported by atmospheric pressure on the open barometer

[1] The modified apparatus was devised by Mr. F. Lyle Wynd, of the Shaw School of Botany, and by the author.

principle as the volumeter, rather than the two burettes of liquid employed by Appleman. With this modification it is possible to make more accurate readings, since the oxygen released in the decomposition of the peroxide is released into a partial vacuum; it does not suffer compression as a result of having to displace the weight of a water column in addition to atmospheric pressure as is the case in the Appleman apparatus.

Seven grams of leaf tissue were ground for 1½ minutes in a chilled mortar with an equal weight of quartz sand and a little powdered calcium carbonate to neutralize the organic acids released by the crushing of the cells. The pulp was then pressed through two layers of cheese-cloth, and 1 cc. of the expressed juice was placed in the reaction bottle (*a*) of the apparatus, which had previously been immersed in a water bath kept constant at 19° C. Then 1 cc. of cold distilled water was added and the stopcock (*b*) closed. Six cc. of 3 per cent C. P. hydrogen peroxide were placed in the funnel (*c*), the stopper (*d*) tightly inserted in the top of the funnel and the water sucked up into the burette (*e*) from the water reservoir (*f*) by suction applied at the rubber tube (*g*). The tube (*g*) was then closed with a stopcock (*h*) and the funnel stopcock (*b*) was opened, allowing the peroxide to run into the reaction bottle (*a*). The evolution of oxygen began immediately, and the water column in the burette immediately began to fall. After the admission of the peroxide to the reaction bottle, the bottle was shaken vigorously and constantly by hand. The evolution of oxygen was allowed to proceed for 2½ minutes, at the end of which the total volume of gas evolved was read on the burette. All glassware, pipettes, mortars, etc. used in the work were chilled in an electric refrigerator before use, since it has been shown that catalase begins to decompose at temperatures slightly above 20° C.

The results of the catalase experiments upon beans and tomatoes are given in table VI.

With the values expressed on the basis of 100 for the unrayed averages, the following comparative figures result:

Beans—unrayed, 100.00; rayed, 143.18.

Tomatoes—unrayed, 100.00; rayed, 149.74.

The figures show that the activity of catalase in the rayed plants is definitely and considerably greater than that in the unrayed plants, a situation comparable to that obtaining in the case of other enzymes tested in these experiments.

TABLE VI
CATALASE
(Cc. of oxygen released)

Experiment number	Beans		Tomatoes	
	Unrayed	Rayed	Unrayed	Rayed
1	13.5	20.0	25.5	37.8
2	16.4	24.4	22.5	34.8
3	15.9	21.2	22.9	33.6
Average	15.26	21.86	23.63	35.4

B. FUSARIUM LINI BOLLEY

For the experiments with this fungus the following culture medium was used:

Magnesium sulphate	1 g.
Calcium phosphate (primary)	1 g.
Potassium nitrate	8 g.
Bacto-peptone	2 g.
Glucose	15 g.
Lintner soluble starch	5 g.
Distilled water	1000 cc.

The fungus was grown in 50 cc. of this medium in 150-cc. quartz flasks. After inoculation the flasks were kept in an incubator at 31° C. for 5 days, a sufficient period to produce a light film of mycelium on the surface of the culture fluid. After this the flasks were set at an angle of 45° in a horizontal rack and were rayed at 10 inches from the arc through a quartz water cell as described in Series I, the same intensity values obtaining in both cases. Exposure periods of varying length were employed in order to find the minimal time which would result in the death of the fungus. After these exposures, the cultures were allowed to remain untouched for an hour. Then transfers were made from them to fresh sterile culture solutions. As long as such transfers resulted in growth, it was obvious that the rayed mycelia were

alive. When, however, no growth appeared in the transfers, it was assumed that the mycelium had been killed by the irradiation. In these experiments, under the conditions mentioned above, an exposure of 12 minutes, with vigorous shaking of the rack at 3-minute intervals, was sufficient to kill the mycelium. After the irradiation the cultures were allowed to stand for an hour before the tissue was prepared for the enzyme tests.

The mycelium mats, torn apart by needles, were poured on to Buchner funnels, washed with several volumes of distilled water, and the excess water removed by pressing the tissue firmly between pieces of filter-paper. The mycelium was then placed in a quantity of 95 per cent alcohol 6 times its own volume and allowed to remain in it for 12 minutes, after which it was placed into a similar volume of acetone, in which it was kept for 8 minutes. Then it was placed in a second volume of acetone for 2 minutes, and after the removal from this bath, was spread out on filter-paper to dry at room temperature. The dried flakes of mycelium were then ground to a fine powder in a mortar. The powder was then placed in tightly stoppered bottles for storage. This powder was used for the determination of amylase and peptase activity.

1. Amylase.—Nine cc. of distilled water were added to 1 gram of the fungus powder in a test-tube, which was then placed in a refrigerator for 12 hours. The juice was then squeezed through double layers of cheese-cloth from the powder-water mixture and was tested by the Wohlgemuth method as described in Series I. The test-tubes containing the starch-enzyme mixtures were incubated for 24 hours at 39° C., after which the iodine test was applied. The results with the amylase experiments together with those of peptase are given in table VII.

2. Peptase.—The activity of peptase was determined by the Sorensen method as described elsewhere in this paper. One and one-half grams of the fungous powder were added to 250 cc. of the 2 per cent Witte's peptone solution in the rayed and unrayed sets, and the determinations made by the thymolphthalein method after an incubation period of 3 days. Blank solutions of powder only and of peptone only were tested as in the work on tomatoes and beans in order to determine the actual amount of amino

acids formed in hydrolysis. The results of the peptase experiments are shown in table VII.

TABLE VII

F. LINI

Experiment number	Amylase D		Peptase Mg. of N formed	
	Unrayed	Rayed	Unrayed	Rayed
1	14.3	16.7	112.7	121.0
2	14.3	16.7	104.1	110.6
3	14.3	14.3	100.2	106.8
Average	14.3	15.9	105.6	112.8

Expressed on the basis of 100 as the value of the averages of the unrayed groups the following numerical comparisons can be made:

Amylase—unrayed, 100.00; rayed, 111.09.
Peptase—unrayed, 100.00; rayed, 106.02.

Hence it seems that there is a slight but consistent increase in the activity of amylase and peptase in the irradiated fungi. The question may be advanced as to whether or not the increases are significant, since they are so small; if these apparent increases are not truly significant, the experiments show at least that the enzymes suffer no decrease in activity when the tissue is killed by ultra-violet rays.

III. Discussion

The inactivation of enzymes *in vitro* by ultra-violet rays has led to the supposition by some physiologists that the injury or death of tissues caused by such rays is attributable in part at least to this inactivation of enzymes. A few reports have been made upon the effects of ultra-violet rays upon enzymes in living plant tissue: those of Pougnet, Stassano and Lematte, Burge, and Green. In so far as the author has been able to determine, these are the only experiments which have investigated the effects of ultra-violet rays upon enzymes in plant tissues.

The results of the experiments reported in this paper which have involved the study of a greater number of enzymes in more

complex tissues agree with those of the above-mentioned earlier works in regard to this fact: namely, it is possible to produce advanced injury (beans and tomatoes) or death (*Fusarium Lini*) in plant tissues without injury to the enzymes contained by those tissues. From this work the assumption may be made that the injury or death of living protoplasm as a result of ultra-violet irradiation is to be ascribed to physiological disturbances other than the inactivation of enzymes.

The present work presents another interesting discovery: that in some cases the activity of enzymes in tissues injured or killed by ultra-violet rays is greater than that of enzymes in healthy, non-irradiated tissues. The possibility suggested itself that a difference in dry weight or in pH of the rayed and unrayed tissues might account for the difference in enzyme activities. Hence, a number of dry-weight and pH determinations of leaf tissue and extracts were made; the results are presented in table VIII.

TABLE VIII

DRY WEIGHT AND pH DETERMINATIONS

Sample no.	Beans				Tomatoes			
	Rayed		Unrayed		Rayed		Unrayed	
	% dry of wet wt.	pH	% dry of wet wt.	pH	% dry of wet wt.	pH	% dry of wet wt.	pH
1	12.0	5.80	12.4	5.80	12.3	6.18	10.3	6.18
2	13.0	5.75	10.1	5.80	11.5	6.18	11.5	6.10
3	12.1	5.81	11.4	5.65	13.2	6.20	12.1	6.18
4	12.2	5.80	10.2	5.85	11.9	6.18	10.7	6.18
5	10.7	5.80	11.8	5.80	12.1	6.10	11.6	6.15
6	11.6		10.9		11.4		10.8	
7	11.4		11.2		10.3		10.1	
8	12.1		11.7		13.5		11.2	
9	11.2		10.1		11.9		10.4	
10	11.0		10.8		12.3		12.0	
Av.	11.83	5.79	10.06	5.78	12.04	6.16	11.07	6.15

The table shows a slightly greater dry weight in the rayed sets both in the cases of the tomatoes and beans. The differences, however, are so small that the increased activity of the enzymes

in the rayed sets can hardly be explained on this basis. The results of the pH determinations show agreement with those of Eltinge ('28), who found no pH differences among rayed and unrayed plants. On the basis of these measurements, then, it must be concluded that the increased enzyme activity is not a result of differences in dry weight or in the pH of tissue extracts.

It was suggested that there might be other secondary ways in which the radiations could have induced such differences in enzyme activity. It is known that in various types of injury to tissues the activity of enzymes, particularly of respiratory enzymes and catalase, is increased. The accelerated activities obtained in these experiments may be such a reaction—merely a stimulation due to tissue injury and not at all a specific reaction to ultra-violet rays. Such an explanation, however, would hardly account for the enormous stimulation obtained in some cases, for example, bean amylase, in which the activity in the rayed tissue is more than 2½ times that in the controls. In such cases, something akin to Pincussen's ('26) reactivation phenomenon may occur. A portion of the enzyme in the tissue may be in a more sensitive condition than another portion and may suffer partial or complete inactivation as a result of irradiation, whereas the second portion may be completely protected by cell proteins, chlorophyll, etc. against the lethal rays. The injured enzyme substance may then through subsequent contact with the uninjured portion experience the reactivation as described by Pincussen in his *in vitro* experiments. Pincussen found that when such unrayed and rayed enzyme solutions were brought together, their combined activity was considerably greater than the summation of their separate activities. Such a phenomenon may account in large degree for the results of the present experiments.

Another suggestion which might be made in regard to the stimulation of enzyme activity concerns the relations between the enzymes and their coenzymes. It might happen that the enzyme itself suffers partial inactivation by the ultra-violet rays and thus leaves an excess of coenzyme. This excess of coenzyme then stimulates the remainder of the actual enzyme to greater activity.

The experiments upon Taka-diastase and Difco invertase illustrate a point made by the author in an earlier paper (Fuller,

'32), in which it was shown that at short distances from a quartz mercury vapor arc the infra-red radiation was responsible in considerable degree for injury previously attributed to ultra-violet radiation. The present experiments upon enzymes *in vitro* demonstrate that infra-red radiation causes a significant proportion of the injury to enzymes rayed in close proximity to a mercury arc, a factor which has been neglected in most of the earlier experiments.

IV. Summary

1. When solutions of Taka-diastase and Difco invertase are irradiated *in vitro* by a mercury vapor arc, the enzymes suffer partial inactivation. This injury is due in part to ultra-violet, in part to infra-red radiations.

2. When bean and tomato plants are severely injured by ultra-violet rays, the activity of their amylase, invertase, peptase, and catalase is significantly increased.

3. When mycelium of *Fusarium Lini* Bolley is killed by ultra-violet rays, there is no decrease in the activity of its amylase and peptase. There is some evidence for a slight increase in the activity of these enzymes.

4. Confirmation is given earlier evidence that the death of living tissue as a result of ultra-violet irradiation is attributable to some factor other than the inactivation of enzymes, and additional data are presented to emphasize those earlier findings.

V. Acknowledgments

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Bibliography

Agulhon, H. ('12). Action de la lumière sur les diastases. Inst. Pasteur, Ann. **26**: 38–47. 1912.

Appleman, C. O. ('10). Some observations on catalase. Bot. Gaz. **50**: 182–192. 1910.

Appleman, C. O. ('11). Physiologic behavior of enzymes and carbohydrate transformations in after-ripening of the potato tuber. *Ibid.* **52**: 306–315. 1911.

Burge, W. E. ('17). The action of ultra-violet radiation in killing living cells such as bacteria. Amer. Jour. Physiol. **43**: 429–432. 1917.

Chauchard, A., et E. Mazoué ('11). Action des rayons ultra-violets sur l'amylase, l'invertine et le mélange de ces deux diastases. Acad. Paris, Compt. Rend. **152**: 1709–1713. 1911.

Downes, A., and T. Blunt ('79). On the influence of light upon protoplasm. Roy. Soc. London, Proc. **B 28**: 199. 1879.

Dreyer, G., et O. Hanssen ('07). Recherches sur les lois de l'action de la lumière sur les glycosides, les enzymes, les toxines, les anticorps. Acad. Paris, Compt. Rend. **145**: 564. 1907.

Eltinge, E. ('28). The effects of ultra-violet radiation upon higher plants. Ann. Mo. Bot. Gard. **15**: 169–240. 1928.

Emmerling, O. ('01). Die Einwirkung des Sonnenlichtes auf die Enzyme. Ber. d. deut. chem. Ges. **34**: 3811. 1901.

Fermi, C., und. L. Pernossi ('94). Über die Enzyme—Vergleichende Studien. Zeitschr. f. Hyg. & Infekt. **18**: 83. 1894.

Fisher, E. R. ('19). Contributions to the study of the vegetable proteases. Biochem. Jour. **13**: 124–135. 1919.

Fuller, H. J. ('32). The injurious effects of ultra-violet and infra-red radiations on plants. Ann. Mo. Bot. Gard. **19**: 79–86. 1932.

Green, J. R. ('97). The action of light on diastase and its biological significance. Roy. Soc. London, Phil. Trans. **188B**: 167–190. 1897.

Hussy, R. G., and W. R. Thompson ('25). The effect of radiations from a mercury arc in quartz on enzymes. I. The effect of ultra-violet radiation on pepsin in solution. Jour. Gen. Physiol. **9**: 217–219. 1925.

Jodlbauer, A., und H. von Tappeiner ('06). Über die Wirkung des Lichtes auf Enzyme in Sauerstoff und Wasserstoffatmosphäre verglichen mit der Wirkung der photodynamischen Stoffen. Deut. Archiv. f. klin. Med. **85**: 386. 1906.

———, ———, ('06a). Über die Wirkung des ultra-violetten Lichtes auf Enzyme (Invertin). *Ibid.* **87**: 373. 1906.

Koeppe, H. ('30). Ultra-violet irradiation and catalase. Archiv f. Kinderheilk. **89**: 1–26. 1930.

Pace, J. ('31). Über die Wirkung von ultravioletten und sichtbaren Licht auf durch Erhitzen inaktivierte Lösungen von Trypsin, Enterokinase und Trypsinkinase. Biochem. Zeitschr. **240**: 490. 1931.

Pincussen, L. ('23). Fermente und Licht I. Diastase I. *Ibid.* **134**: 459. 1923.

———, und N. Kato ('23). *Ibid.* II. Urease I. *Ibid.* 470. 1923.

———, ———, ('23a). *Ibid.* III. Urease II. *Ibid.* **142**: 228. 1923.

———, und F. di Renzo ('24). *Ibid.* IV. Diastase II. *Ibid.* **144**: 366. 1924.

———, ('24). *Ibid.* V. Diastase III. *Ibid.* 372. 1924.

———, ('24a). *Ibid.* VI. Über Beeinflussung von Fermentwirkungen durch Jodsalze unter Bestrahlung. *Ibid.* **152**: 406. 1924.

———, und N. Klissiunis ('24). *Ibid.* VII. Über Beeinflussung von Fermentwirkungen durch Jodsalze unter Bestrahlung, II. *Ibid.* 416. 1924.

Pincussen, L., und F. Seligsohn ('26). *Ibid.* VIII. Katalase I. *Ibid.* **168**: 457. 1926.
———, ('26). *Ibid.* IX. Diastase IV. *Ibid.* **171**: 1. 1926.
———, und S. Kumanomidoh ('28). *Ibid.* X. Diastase V. *Ibid.* **195**: 79. 1928.
———, und K. Uehara ('28). *Ibid.* XI. Pepsin I. *Ibid.* 87. 1928.
———, und S. Hayashi ('28). *Ibid.* XII. Lipase I. *Ibid.* 96. 1928.
———, und Y. Kambayashi ('28). *Ibid.* XIII. Die Wirkung des Lichtes auf Taka-diastase bei Zusatz von Sensitasoren. *Ibid.* **203**: 334. 1928.
———, und T. Oya ('29). *Ibid.* XIV. Versuche über den Einfluss der Temperatur der Lichtwirkung. *Ibid.* **207**: 410. 1929.
———, ———, ('29a). *Ibid.* XV. Über die Spaltung des Lecithins durch die Lecithase und Phosphatase des Preparates "Taka-diastase." *Ibid.* **215**: 367. 1929.
———, ———, ('29b). *Ibid.* XVI. Über das pH Optimum der Milchaldehydrase und die Beeinflussing dieses Fermentes durch Licht. *Ibid.* 398. 1929.
———, und W. Roman ('30). *Ibid.* XVII. Über den Einfluss des sichtbaren und ultra-violetten Lichtes auf die Succinodehydrogenase des Pferdemuskelfleisches. *Ibid.* **229**: 281. 1930.
Pougnet, J. ('10). Action des rayons ultraviolets sur les plantes à coumarine et quelque plantes dont l'odeur provient de glucosides dédoublés. Acad. Paris, Compt. Rend. **151**: 566–569. 1910.
———, ('11). Action des rayons ultraviolets sur les gousses vertes de vanille. *Ibid.* **152**: 1184–1186. 1911.
Schmidt-Nielsen, S. ('04). Die Enzyme namentlich das Chymosin, Chymosinogen und Antichymosin in ihrem Verhalten zu konzentriertem elektrischem Lichte. Hofmeister's Beiträge **5**: 355. 1904.
———, und S. ('08). Quantitative Versuche über die Destruktion des Labs durch Licht. Zeitschr. f. physiol. Chem. **58**: 233. 1908.
Sherman, H. C., E. C. Kendall and E. D. Clark ('10). Studies on amylases; I. An examination of methods for the determination of diastatic power. Amer. Chem. Soc., Jour. **32**: 1073. 1910.
Sorensen, S. P. ('08). Enzymstudien I: Über die Quantitative Messung proteolytischer Spaltungen. Biochem. Zeitschr. **7**: 45. 1908.
Stassano, H., et L. Lematte ('11). De la possibilité de conserver intactes les agglutinines dans les bactéries qu'on tue par les rayons ultraviolets. Avantage de ce moyen de stérilisation pour préparer les emulsions bactériennes destinées aux sérodiagnostics. Acad. Paris, Compt. Rend. **152**: 623–624. 1911.
von Tappeiner, H. ('03). Über die Wirkung fluorescirender Substanzen auf Fermente und Toxine. Ber. d. deut. chem. Ges. **36**: 3035. 1903.
Tauber, H. ('30). Studies on crystalline urease. Inactivation by ultra-violet radiation, sunlight with the aid of a photodynamic agent, and inactivation by trypsin. Jour. Biol. Chem. **87**: 625. 1930.
Thompson, W. R., and R. Hussey ('31). The effects of radiations from a mercury arc in quartz on enzymes. II. The effect of ultra-violet radiation on amylase in solution. Jour. Gen. Physiol. **15**: 9–13. 1931.
Waksman, S. A., and W. C. Davison ('26). Enzymes: Properties, distribution, methods, and applications. 364 pp. London, 1926.
Wohlgemuth, J. ('08). Ueber eine neue Methode zur Quantitativen Bestimmung des diastatischen Ferments. Biochem. Zeitschr. **9**: 1–9. 1908.

GENERAL INDEX TO VOLUME XIX

New scientific names of plants and the final members of new combinations are printed in **bold face** type; synonyms and page numbers having reference to figures and plates, in *italics;* and previously published names and all other matter, in ordinary type.

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